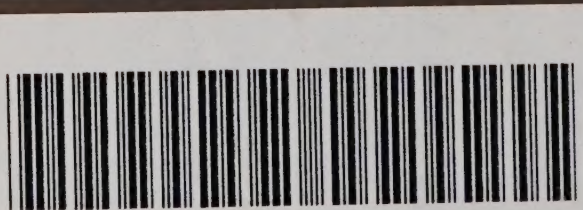
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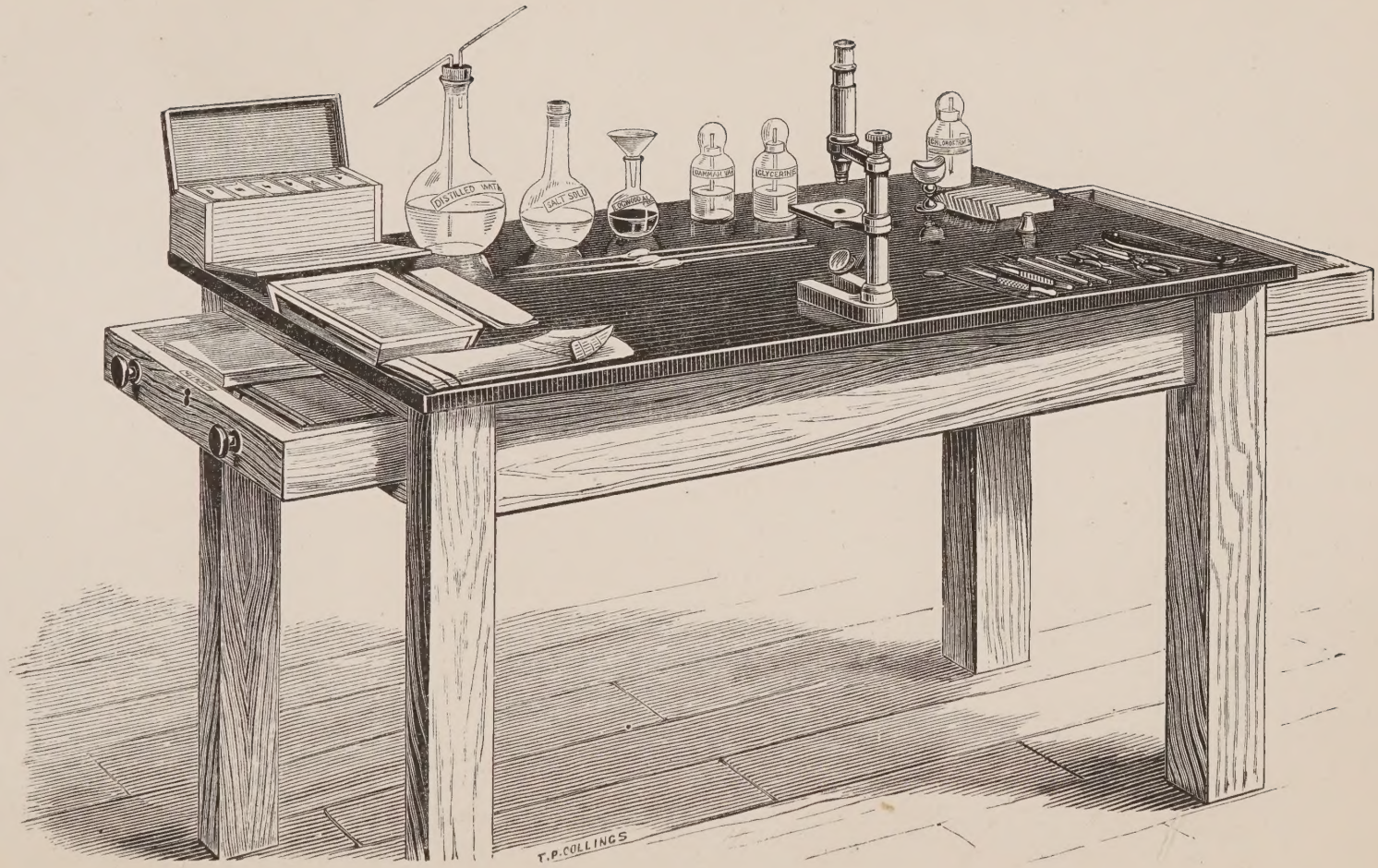

ARRANGEMENT OF WORKING TABLE.

A COURSE

OF

PRACTICAL HISTOLOGY

BY

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LONDON
SMITH, ELDER, & CO., 15 WATERLOO PLACE
1877

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PREFACE.

THE PURPOSE of this work is to afford to those engaged in the practical study of Histology, plain and intelligible directions for the suitable preparation of the animal tissues; with the object either of immediate study, or of their preservation as specimens for future reference. The methods recommended have all been tested by experience.

In an introductory chapter an account is given of the several parts of the microscope, and the purpose for which they are intended, without entering into an explanation of its optical construction; and, in an Appendix, instructions will be found for measuring and for delineating microscopic objects.

Throughout the book descriptions of tissues have been purposely avoided, seeing that these are to be found in systematic works. The order followed is that of 'Quain's Anatomy,' 8th Edition.

With the exception of Figure 12, which is taken from Dr. Burdon Sanderson's 'Handbook for the Physiological Laboratory,' the illustrations have been prepared expressly for this work: they are from the pencil of Mr. Collings.

The methods employed in the practical study of Embryology have been omitted: they will be found admirably given in the 'Elements of Embryology' of Dr. M. Foster and Mr. F. M. Balfour.

University College, London:
November 1876.

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Errata.

Page 65, lines 8 and 12 from bottom of page, *for* extensor *read* flexor.

„ 81, line 6, *for* less *read* more.

PRACTICAL HISTOLOGY.

INTRODUCTORY.

THE practical study of Histology is mainly dependent upon the use of the microscope. The microscope is a combination of lenses arranged for the purpose of obtaining and viewing a magnified image of any minute object. The lenses are set in a tube of variable length—*the tube of the microscope* (fig. 1, t, t')—and this is itself supported in a vertical position, on a firm, metal *stand*, which is provided with an arrangement by which the tube is capable of being moved, without lateral deviation, in a perfectly straight, up and down direction. This arrangement is termed the *adjustment*. Its purpose is to bring the microscope into that position with regard to the object in which the latter is most clearly seen. The object is then said to be in focus.

Two adjustments are commonly provided; one—the *coarse adjustment* (*adj*)—serves to bring the lenses roughly into the focal position, and is either a telescopic joint or a rack and pinion movement; the other—the *fine adjustment* (*adj'*)—is a fine screw, and by its means the focus may be obtained with complete exactness even when the highest magnifying powers are employed. The stand is further provided with a rigidly connected, horizontal table or stage (*st*), upon which the object is placed, and which projects below the tube and is provided

FIG. 1.

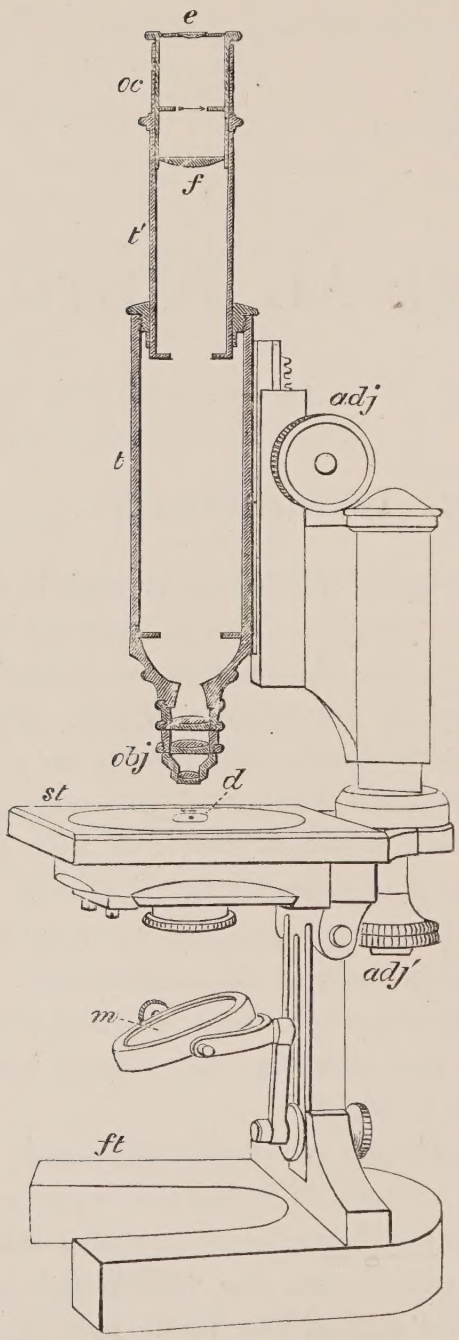


Diagram of microscope.

t, tube seen in section; *t'*, sliding part of tube; *oc*, ocular; with *e*, eye-glass, and *f*, field-glass; *obj*, objective; *adj*, coarse, and *adj'*, fine adjustment; *st*, stage; with *d*, diaphragm; *m*, mirror; *ft*, foot of microscope.

The object is indicated by a small arrow just above the aperture in the diaphragm; the magnified image by the larger arrow in the middle of the ocular.

with a circular aperture to admit light from below to the object, capable of being varied by means of a diaphragm (*d*) furnished with holes of different sizes. Diffused daylight is, if possible, employed, and is reflected, by means of a movable *mirror* (*m*) below the stage, up through the object and through the tube of the microscope to the eye of the observer. This is termed viewing an object by transmitted light. Occasionally, especially when comparatively large and opaque objects and low magnifying powers are to be employed, the former are viewed by the light which is reflected from their surface, whilst that from the mirror is cut off. In order in such cases to concentrate as much light as possible upon the object a bull's-eye condenser is employed. It is only in viewing such preparations that the binocular microscope offers any material advantage in histology.

The lenses form the essential part of the microscope, and are so arranged that one set of them, which is placed at the lower end of the tube, produces a magnified, inverted image of the object at the upper part. The image thus produced is viewed, and at the same time still further magnified, by a lens at the top of the microscope tube. This lens (*e*), placed close to the eye of the observer, is fixed in the *eye-piece* or *ocular*; but this part includes another glass (the *field-glass*, *f*) situate below the first and fixed in one piece with it, having for its object the collection of the more divergent rays transmitted by the lower set of lenses, and serving also to lessen or obviate any chromatic aberration which might otherwise be produced. The whole eye-piece (*oc*) thus composed is made to slip in at the superior aperture of the microscope tube. The lower set of lenses form an achromatic combination which, from its situation near the object, is termed the *object-glass* or *objective*, and it is upon the perfection of its construction that the usefulness of the microscope mainly depends. It is seldom necessary to have more than one ocular of medium strength in use, but at least two objectives of different magnifying powers are essential for histological work. One of

these, which in subsequent pages will be spoken of as the *low power*, should, when used with the ordinary eye-piece, give an apparent linear enlargement of about 75 (75 diameters); that is to say, when a line, the length of which is known (say $\frac{1}{100}$ of an inch), is observed through this combination it should appear seventy-five times as long as it really is ($\frac{3}{4}$ inch therefore). The other, to be mentioned as the *high power*, should give an apparent linear magnification of from 300 to 400 times. These two glasses amply suffice for all ordinary histological studies; but for certain special subjects it is advantageous to obtain the use of a more powerful combination; one that will magnify 1,000 diameters or more.

Glasses of this high magnifying power are usually of the kind known as 'immersion-objectives,' so-called because they are made so as to be used with a stratum of *water* between the specimen and the lower lens of the objective. The water (distilled) is applied to this lens with a splinter of wood or from a pipette before the objective is screwed on to the tube of the microscope; the tube is then lowered by means of the coarse adjustment until the drop of water comes in contact with the cover-glass, after which the focus is obtained by cautiously lowering it further, at first with the coarse, and then with the fine adjustment.

These comprise the essential requirements of a microscope, but larger instruments are often provided with certain adjuncts which render them in a measure more complete. Thus the stand may be hinged so that the stage and tube can be tilted somewhat out of the perpendicular to allow of better adaptation to the position of the observer. But if the microscope tube is not inconveniently high, it is almost as comfortable to work without inclining the instrument. Moreover, very many preparations, most of those, for instance, which have to be examined in fluid, will not admit of inclination. A camera lucida (one which does not necessitate the tilting of the microscope) is useful for obtaining an exact sketch of the outlines of an object.

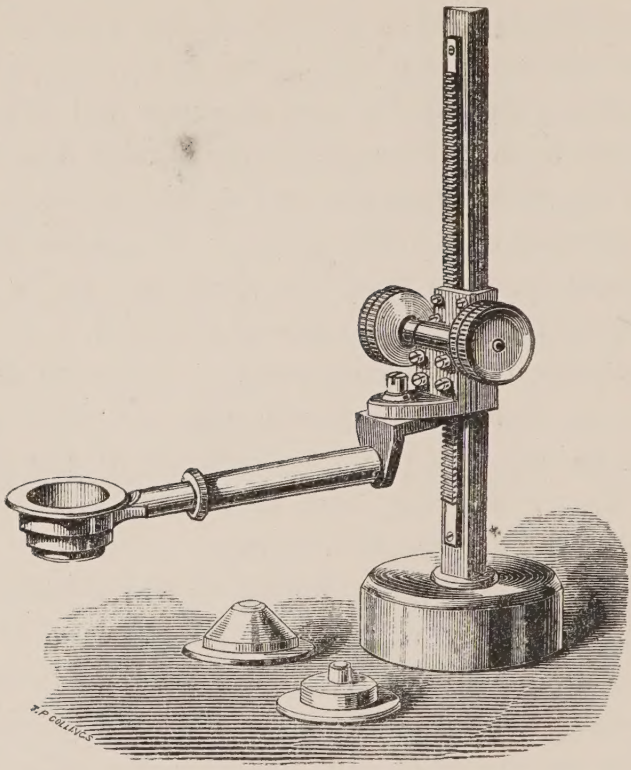
A polarisation apparatus is occasionally employed in investigating the optical properties of the substances which compose the tissues. (It is also of use in helping to determine the nature of crystalline deposits in urine and other fluids.) In connection with the employment of this the stage-plate and tube of the microscope are, in the best instruments, made capable of rotating on a vertical axis. In smaller instruments this movement is, as a rule, not provided for, and indeed, although convenient, is by no means essential.

The mechanical stage movement, which is so often fitted to microscopes of English construction, and other appliances which tend to mar the perfect simplicity of the instrument when in ordinary working trim, serve rather to detract from its usefulness for purposes of histology.

Attention to the foregoing points, of which perhaps perfect steadiness and rigidity of the stand and stage is the most important to insist upon, will aid the student in selecting a useful microscope so far as the body of the instrument is concerned. The excellence of the objectives can only be competently judged of by one who is already somewhat conversant with the use of the microscope. Preparations of some of the tissues or fluids of the body (connective tissue, blood, salivary corpuscles) form the best test objects for the high powers of an instrument that is to be employed in histological studies.

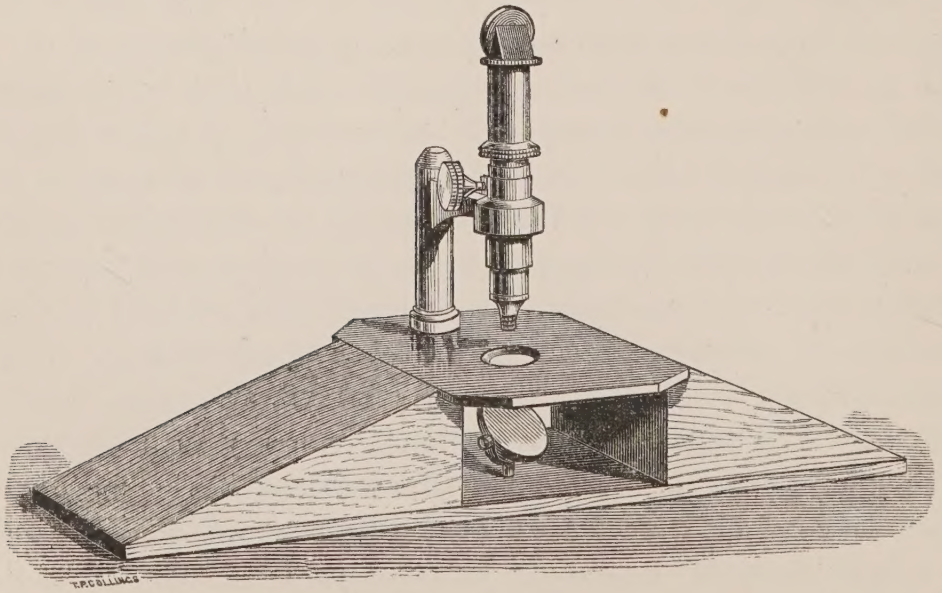
In addition to the ordinary instrument (which is generally distinguished as the 'compound' microscope), it will be found very convenient to have a smaller microscope of some sort: so as to be better able to follow the needles or other instruments when the operator is engaged in the separation and manipulation of minute objects. An instrument which is used for this purpose is termed a dissecting or preparing microscope. Any simple lens which is mounted on a stand will serve (fig. 2), and even the bull's eye condenser, which is generally furnished with the microscope, may be employed as a lens if the marginal part is covered by a black-paper diaphragm. A very convenient form of dissecting microscope is shown in fig. 3.

Fig. 2.



Simple form of dissecting microscope, provided with three lenses of different magnifying power.

Fig. 3.



This consists of a small compound microscope furnished with an arrangement for reversing the image, so that the object appears in its natural position and not inverted. It is provided also with a prism for reflecting the light in a direction convenient for the eye, and is placed on a wooden stand so constructed as to afford support to the arms of the operator. The ordinary low power objectives may be used with it.

Besides the microscope, the student who is commencing the practical study of histology will find it necessary to be provided with the following simple instruments and appliances :—

Glass-slides and cover-glasses (see fig. 5).—The microscopic slides are oblong slips of glass upon which the object is placed. In this country they are always cut of the uniform and convenient size of three inches by one inch. The glass should be quite free from flaws and specks. The cover-glasses can hardly be too thin. It is true that when very delicate they are likely to be broken in the cleaning unless great care is exercised ; but then, on the other hand, many high power objectives require to be focussed so close to the object that a thick cover-glass cannot be used without risk of crushing the tissue, and perhaps of scratching the objective. As a rule, the chief difficulty in cleaning them arises from the fact that their surfaces are generally covered with a thin film of grease or other organic matter which it is almost impossible to rub off. But this is easily got rid of by placing a number of them together in a small glass beaker and pouring a little strong nitric acid upon them. This quickly destroys every trace of organic matter. The acid is then poured off, and the cover-glasses are thoroughly rinsed by allowing water to stream over them from a tap for two or three minutes. They are then to be kept in water ready for use, and need only be dried when wanted. The drying is effected by a thin linen cloth, one corner of which is laid flat on the table, and the cover-glass, having been placed on this, is gently rubbed, first on the one surface and then on the other, with another corner of the cloth. Square cover-glasses should always be

used in preference to round ones. The most convenient size for general purposes is $\frac{3}{4}$ inch square.

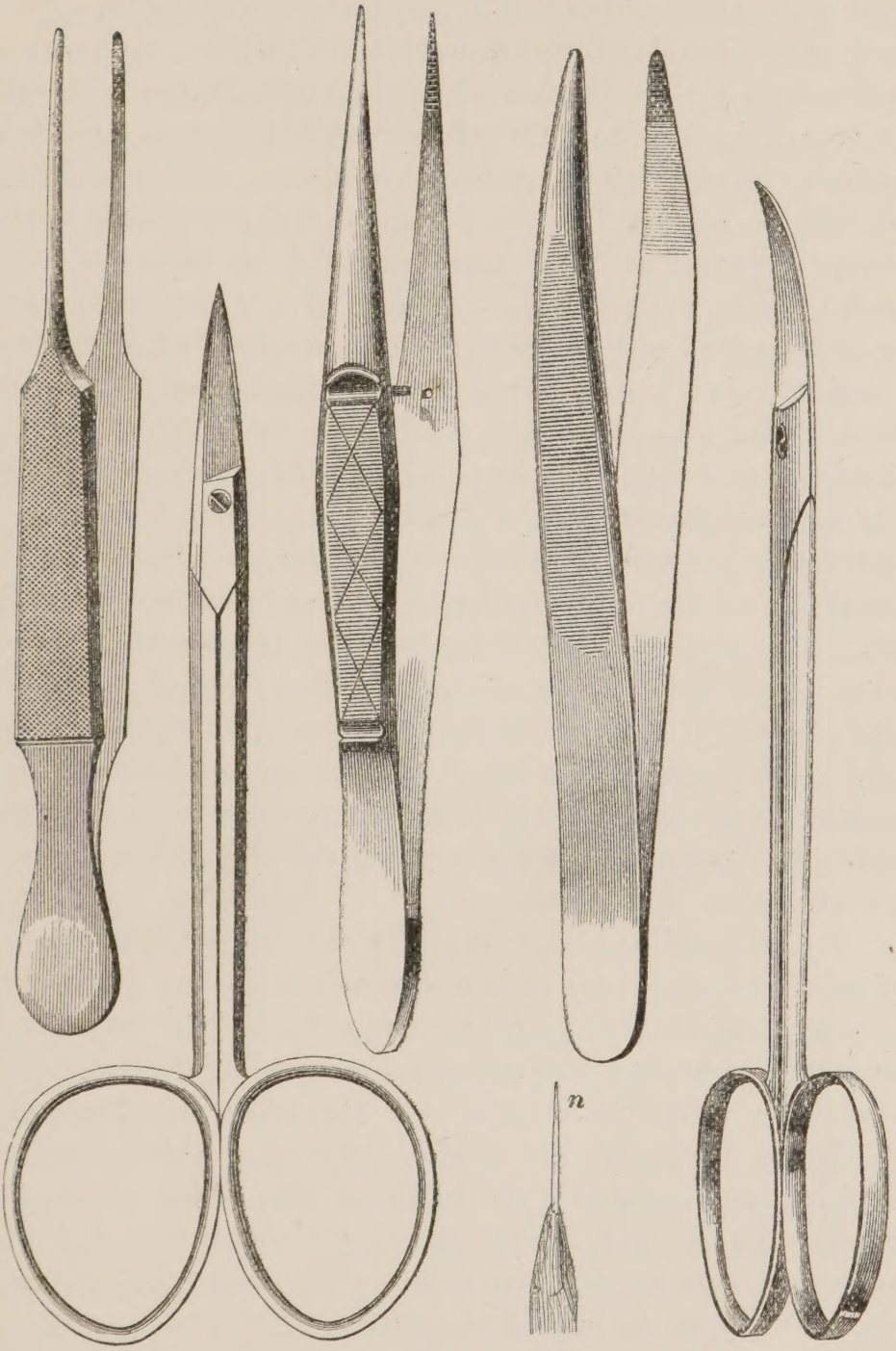
Mounted needles.—These are fine sewing-needles mounted in a wooden handle, with about a quarter of an inch of their point projecting. They are amongst the most useful instruments which the histologist possesses, and will be in constant requisition. They must always be kept clean and sharp. Needles may also be mounted in crochet-needle holders. These have the advantage that fresh needles can at any time be readily substituted.

Scissors and forceps.—A small pair of scissors with short, straight blades, is necessary. Their cutting edge must always be kept very sharp, especially towards the point, otherwise they are worse than useless. A small, curved pair is often useful, but these are much more difficult to keep sharp. At least two pairs of small steel forceps are requisite. The blades should be short, broad at their junction, so as not to admit of lateral deviation, and towards the end gradually tapering to a blunt point. They are slightly roughened at the end, so as to afford a firmer grasp. The long, slender forceps which are commonly sold as microscopic forceps are useless for histological purposes.

Slips of white bibulous paper should always be at hand. They serve both for soaking up excess of fluid from under the cover-glass and for placing the slide upon, when preparing a tissue that has been stained, so that it is better seen than it would be upon a black surface. On the other hand, it is better to use the black surface for working upon when tissues are unstained. One or two large, *shallow glass dishes*, either flat (such as are used by photographers) or watch-glass shaped, are in constant requisition, since very many of the manipulations require to be performed whilst the tissue is immersed and floated out in fluid; while for applying reagents to a specimen under the microscope small pipettes are extremely convenient.

Reagents.—Two or three small bottles containing the fluids

FIG. 4.



Scissors and forceps suitable for histological purposes. Natural size.

n, end of a mounted needle.

which are most used for preserving and mounting preparations should be always on the work-table. Small bottles provided with a cap and with a piece of glass tube drawn out to a point and standing in the bottle are perhaps the best, but small corked phials with a piece of glass tube passed through the cork will answer every purpose. One of these should contain a mixture of glycerine and water (equal parts of each); another a solution of Canada balsam in chloroform for fixing the cover-glass when preparations have been mounted in glycerine (this should have a small brush instead of a glass tube); and another, which will not be used much at first but will afterwards be often in requisition, should contain dammar varnish. It is useful also to have a wash-bottle of distilled water, a flask of salt solution, a solution of chloride of sodium, containing 1 part of the salt to 150 or 200 of water, and a small flask of recently prepared logwood alum staining solution. This is made by taking 5 grammes each of powdered alum and extract of logwood, and rubbing them up thoroughly together in a mortar with 100 cc. of water. The mixture is covered and allowed to stand overnight, it is then filtered into a bottle and a few drops of a solution of peroxide of hydrogen are added. The solution will keep two or three weeks in good condition for staining, but it must always be re-filtered immediately before being used.

Pipettes may be readily made by drawing out in the flame a piece of soft glass tube at two places close to one another, so that the intermediate part remains as the bulb of the pipette. It is well to make a number at a time, sealing up the ends in the flame while the bulb is still hot; they are thus made absolutely dust-tight and may be kept always ready for use, it being only necessary to break off the sealed ends when required and to suck the reagent up into the bulb. A pipette should always be rejected after it has once been used, and never employed for another reagent.

For keeping preparations that have been permanently

mounted, it is desirable to have a box or cabinet in which the slides can be put away in a horizontal position.

General directions for work.—Before commencing see that the table is in order and clean, and that everything is at hand that is usually wanted for histological work. Especially look carefully to the glasses of the microscope that there is no dust or other impurity on them. If any glycerine or Canada balsam should have found its way on to the objective, as is often the case when sufficient care is not taken in placing a preparation upon or removing it from the stage, they are to be rubbed off, the former by a cloth wetted with water, the latter with a little spirit. Before beginning to prepare a tissue it will be necessary to look over the description of the mode of making each preparation, in order to know what vessels and what reagents to get together. Otherwise many a specimen will be spoiled by being left too long in one fluid whilst the one to which it should be transferred is being got ready. The cover-glass should always be cleaned and dried before commencing, and placed ready to hand in some situation where it is not likely to get broken. It is well always to put the cleaned cover-glasses in the same place—say, on the foot of the microscope—otherwise, when wanted in a hurry it is often difficult to find them at once.

Every specimen that is to be kept must be distinctively labelled as soon as made; and if there is anything of importance to remember about it this must be at once entered in the notebook, which no one who is working with the microscope should be without.

Lastly, the student should never trust to the transient impression of form or structure which the mere glance at a microscopic preparation conveys, but should always, whether naturally a good draughtsman or not, endeavour to perpetuate the impression so obtained by a careful sketch showing the more important points which the preparation illustrates. The rough outlines may be drawn with a camera lucida or drawing

prism, if one be available, but even without such an instrument a little practice soon enables a sketch to be produced which gives a fairly good idea of the appearances seen, and however rough it may be, serves materially to assist the memory.

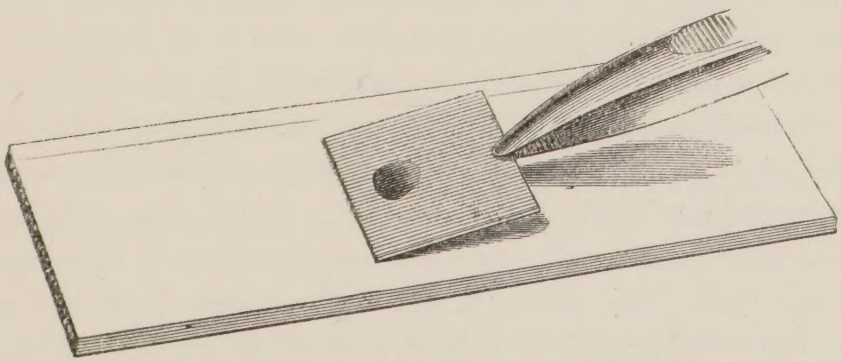
For the most part it is also desirable to note the results of measurements made with a micrometer. The use of this, as well as the method of sketching an object by aid of the camera lucida, will be described in the Appendix at the end of the book.

CHAPTER I.

THE BLOOD.

Preparation 1.—A drop of blood may be most conveniently obtained for examination from the finger. It is generally sufficient to give the end of the forefinger of the left hand a smart prick with a clean sewing-needle, in the thin part of the skin adjoining the root of the nail, squeezing firmly with the right hand above the point pricked, to cause a drop to exude. If necessary, the finger may first be congested by tying a piece of string tightly round it. As soon as a small drop of blood has been pressed out, take up a previously cleaned cover-glass by one edge with forceps, let the drop come in contact with the lower surface of the glass near the opposite edge, so that a little adheres; and then, letting this edge come first in contact with the upper surface of the slide

FIG. 5.



Glass slide and cover-glass, natural size.

The figure shows the mode of letting down the cover-glass (with a drop of blood on its under surface) gently on to the middle of the slide.

near its middle, gradually lower the other edge, which is still held in the forceps, on to the slide (fig. 5). When

the lower blade of the forceps nearly touches the slide, withdraw the instrument carefully, so that the cover-glass may now rest evenly upon the slide by its whole under surface, with the blood in a uniform thin layer between. It is important not to let the cover-glass down too suddenly, for if dropped carelessly on the slide, many of the corpuscles will be broken and destroyed. When the cover-glass is in its place, there ought to be just enough blood *entirely* to fill the space between the two glasses; but it is better to have too little than too much. It might be supposed that the delicate corpuscles would be crushed between the cover-glass and slide, but they are for the most part protected from this by the buoying-up of the cover-glass by the liquid in which they float.

The preparation made, it is to be at once transferred to the stage of the microscope, and examined with a power of about 300 diameters. The field of the microscope will be seen crowded with corpuscles floating in a clear liquid. Probably the first thing which will strike the beginner is the very faint colour which the so-called *red* corpuscles present, and these will also most likely be the only kind of corpuscle that he will at first be able to distinguish. But if at the moment of observation there happens to be a current in the fluid—produced either accidentally by a shaking of the room, or a draught of air, or purposely by gently touching the cover-glass with a bristle—it will be seen that while most of the corpuscles are carried along by the current, two or three remain sticking to the glass, whilst the others are carried past them; and, on close examination, it will further be clear that these are of a different nature from the rest, being entirely devoid of colour, and of a pale granular appearance. They are in fact white corpuscles, and once seen, will be easily recognised again even when the fluid is at a standstill.

Another thing that will be made manifest by any motion in the fluid is the biconcave discoid shape of the red corpuscles,

for as they roll over it will be seen that their outline is no longer circular as when lying flat, but on the contrary, a lateral view of the disks is obtained, and the flattening and incurvation of the surfaces become evident.

When the motion in the layer of blood, in whatever way it may have been produced, is subsiding, it will be seen that whenever one corpuscle comes in contact with another the two seem to be in some way attracted to one another, so as to adhere closely by their opposed surfaces; and other corpuscles coming in the same way in contact with these and adhering, little piles or rouleaux are thus produced, which form by their junction with one another a network, extending throughout almost the whole of the preparation. In the cords of this network nearly all the red corpuscles are involved, and now for the most part are seen edgeways; but in other parts of the preparation where the layer of blood is very thin —the space being too small to allow the corpuscles to stand edge up, and to combine so completely to form rouleaux—they may be found still lying flat and distinct from one another; and these more isolated corpuscles may now be subjected to careful examination. Keeping a single red corpuscle in view, if it be brought exactly into focus—that is to say, if the microscope be so adjusted that the contour of the corpuscle is as distinct as possible—it will be observed, with the power (300 diameters) which is at present being employed, that the middle part appears slightly darker than the rim, whereas if, by means of the fine adjustment, the objective be now brought somewhat *nearer* (lower), the middle part will come to appear lighter.

The cause of this is probably to be found in the *shape* of the corpuscle, the middle part of which acts upon the light like a very weak biconcave lens, refracting the rays of light which are transmitted through it slightly outwards; so that if the objective is at a certain distance, all of the rays which traverse the central part do not reach it, some of them being deflected too much to the side to impinge upon the lower glass of the objective. The part in question, therefore,

looks a little dimmer than the somewhat convex marginal part, whereas when the objective is brought *nearer* all the rays are intercepted by it; and the middle part, owing to its greater thinness, appears lighter than the rim. If a very high power objective is used the middle part of the corpuscle will be the lighter, even when the focus is rightly adjusted, for in these objectives the focal length is generally very short, and they approach, therefore, near enough to intercept the outwardly refracted rays.

With the exception of these differences of shading (which are merely dependent upon the shape of the corpuscle), the red corpuscles present a perfectly homogeneous appearance, and exhibit in the fresh condition no tendency to separate into the two parts of which, as the study of the action of reagents will show, they in reality consist. But there may generally be noticed, even in a preparation which has been made with the greatest care, a red corpuscle here and there which varies from the prevailing form, having become more globular, and at the same time rather smaller in diameter: some of these retain a smooth contour, whilst others, especially those near the edge of the preparation, have a jagged or crenate margin, as if set with little projections, and these may also be seen on the surfaces of the corpuscles by carefully adjusting the microscope. This change of form, which is very characteristic of the mammalian red corpuscles on exposure, seems to be generally caused by a shrinking of the corpuscles, induced by an increase in the density of the plasma in which they float: it may always be produced by adding salt to blood.

Turning now our attention to the white corpuscles, not more than two or three of which are to be seen in each field of the microscope when the ordinary high power is being used, and which, as before stated, are readily distinguishable from the red corpuscles by their want of colour and their pale, granular aspect, we usually notice, if the room is moderately cool, and provided they are not pressed down by the cover-glass, that they are spheroidal and completely

motionless, exhibiting no indications of vitality. Some of them may be noticed to contain a small group of well-marked granules, much coarser than the excessively fine granules which pervade the whole substance; and in conformity with this it is usual to describe two kinds of white corpuscles—the finely granular, and the coarsely granular—but there would not seem to be any very essential difference between the two. As a rule, before the addition of reagents, no nucleus is visible in either variety, although, as will be afterwards seen, one or more is always present in each; the nuclei are delicate, however, and readily obscured by the granules of the protoplasm. If the room is tolerably warm it may happen that the white corpuscles no longer preserve their rounded outline, but that from one side or another of a corpuscle a bud-like process extends itself, to be again retracted into the body of the corpuscle; spontaneous changes of form being thus effected which resemble those which are presented by the common fresh-water amœba, and are hence termed 'amœboid;' but in a cold preparation of human blood, like that under examination, these movements are seldom extensive, and do not serve to effect an actual change of place in the corpuscles such as we shall see to be the case in a preparation which is artificially warmed.

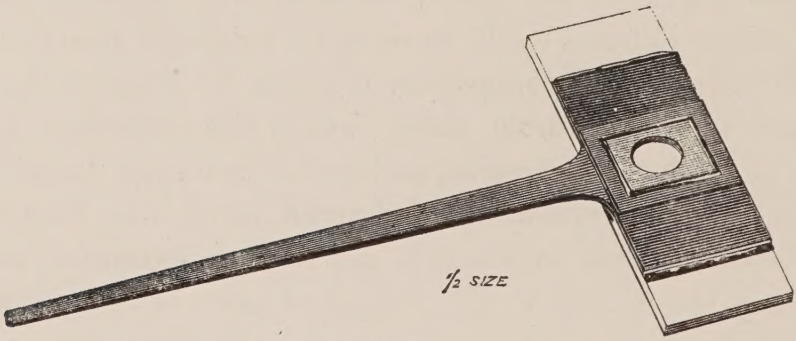
Further, there may generally be seen in a preparation of blood a number of excessively minute pale granules, which, if present in quantity, may be closely grouped together here and there into masses or 'colonies' of various shapes and sizes (see fig. 8, *a*), which the beginner is sometimes apt to mistake for white blood-corpuscles. But the objects in question have a much fainter aspect; and although under certain conditions they—at least, their constituent granules—may, as we shall presently see, exhibit indications of vitality, yet nothing resembling in nature the amœboid movements of the white blood-corpuscles is ever observed in them.

Finally, a few excessively fine and delicate threads of fibrin may be observed stretching in different directions

across the field of the microscope; but to see these distinctly a very high power is needed.

Preparation 2.—In order properly to study the vital phenomena which are displayed by the white blood-corpuscles it is necessary, in the case of man and warm-blooded animals, to maintain the drop of blood under observation at or near the temperature of the body. For this purpose we employ what is known as a warm stage, of which there are several forms in use. The simplest consists merely of an oblong copper plate (fig. 6), two inches by one inch, from one side of which a rod of the same metal, four or five inches long, projects. This plate has a round aperture in the middle, half

FIG. 6.



Simple warm stage, with preparation upon it enclosed between two cover-glasses.

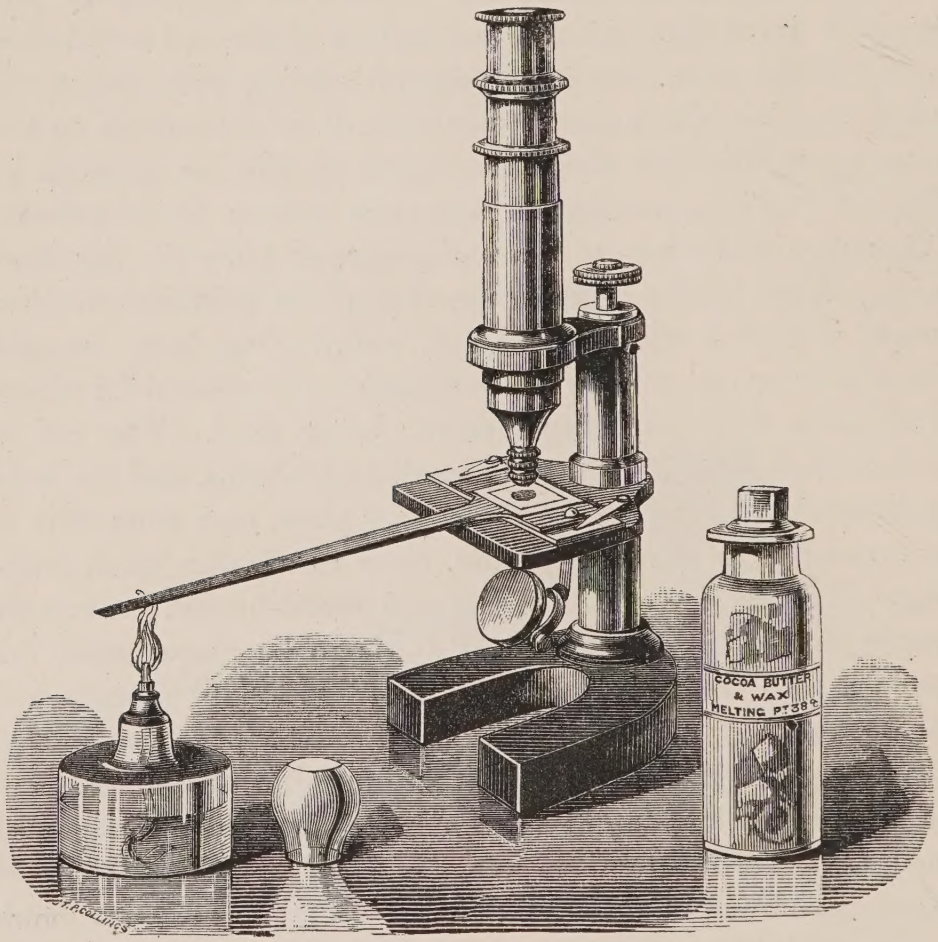
an inch in diameter, and is fastened to an ordinary slide by sealingwax. The preparation is made as follows:—Take first a clean, large-sized (one inch square) cover-glass, which in this case is to be used instead of a slide, and on it place a small drop of salt solution. With this mix thoroughly with a needle about an equal amount of blood obtained from the finger, as in Prep. 1, and carefully cover the mixed fluid with another cover-glass, somewhat smaller than the first. If there is now not enough fluid to fill the space between the two glasses, add a little more salt solution at one edge of the smaller cover-glass; but if, on the other hand, there is too much, soak up the excess with a small piece of blotting-paper.

A very small camel-hair pencil which has been dipped in olive-oil is now to be drawn gently along each edge of the smaller glass; this will prevent evaporation from the edges, which would otherwise quickly ensue on warming the preparation. The dilution of the blood with salt solution prevents in great measure the aggregation of the red corpuscles, while at the same time in no way interfering with the movements of the white ones; moreover, it is favourable to the changes which the above-mentioned masses or colonies of discoid particles undergo, if any such happen to be present. The glass slide which bears the copper plate having been clamped on to the microscope stage, the preparation thus made is placed upon the copper, and, having been brought in focus, one or more white corpuscles are selected for observation—a high magnifying power being used. The rod is now heated near its end by a small spirit-lamp, and the heat is conducted by the rod to the copper plate, and from this is transmitted to the preparation, close to which a small fragment of a mixture of white wax and cacao-butter, previously made, and melting at about 30° C., is to be placed upon the copper (fig. 7). The lamp is now gradually approached along the rod until it arrives at a spot the heat transmitted from which is just sufficient partially to melt the fragment, and it is then left burning at that spot, for since the fat employed melts at about the temperature of the body, we know that the preparation will now be also warmed nearly to the same point.

It will be seen that as the preparation begins to get warm the white corpuscles, which were perhaps previously rounded and inert, begin to throw out processes and exhibit amœboid movements, which become more and more marked as the temperature rises, so that by virtue of these an actual change of place from one part of the field to another may be effected. It is well in making this observation to select a single corpuscle and to sketch its outline and that of its more immediate surroundings at intervals of half a minute. As the corpuscles become spread out in creeping along the

glass one or more nuclei may sometimes be seen indistinctly in them; more often, perhaps, clear spaces or vacuoles are to be seen in their protoplasm.

Fig. 7.



Simple warming apparatus, complete, shown in operation.

The red corpuscles in this preparation may be disregarded, for they show no trace of amœboid movement, but only become slightly crenate, owing to the action of the salt solution. The slight shaking movement which many of them exhibit is the molecular or Brownian movement common to all minute solid particles floating in a liquid.

The changes which the colonies of discoid particles (see p. 17) undergo under the present conditions—*i. e.* dilution of the blood with

salt solution and warmth—should be carefully studied. As a rule, the larger the colony the more actively the changes, about to be described, take place. If there are none to be seen in the blood from the finger a drop may be obtained from some other source and prepared in a similar way. They are common in the blood of some animals—*e. g.* the rat—and are generally large and numerous in that of persons who have been long ill.

The masses in question (fig. 8, *a*) are often of considerable size, many times larger than a pale blood-corpuscle. As Osler has shown, the particles which compose them are free in the circulating blood, and only run together when the blood is drawn.

About half an hour after the preparation has been made the masses may be seen to have no longer an even, tolerably well-defined contour, but to be bristling with excessive minute filamentous projections, each of which has grown out from one of the minute discoid particles at

FIG. 8.

Changes seen in one of the masses or colonies of discoid particles from a drop of human blood, diluted with salt solution and warmed. After Osler.

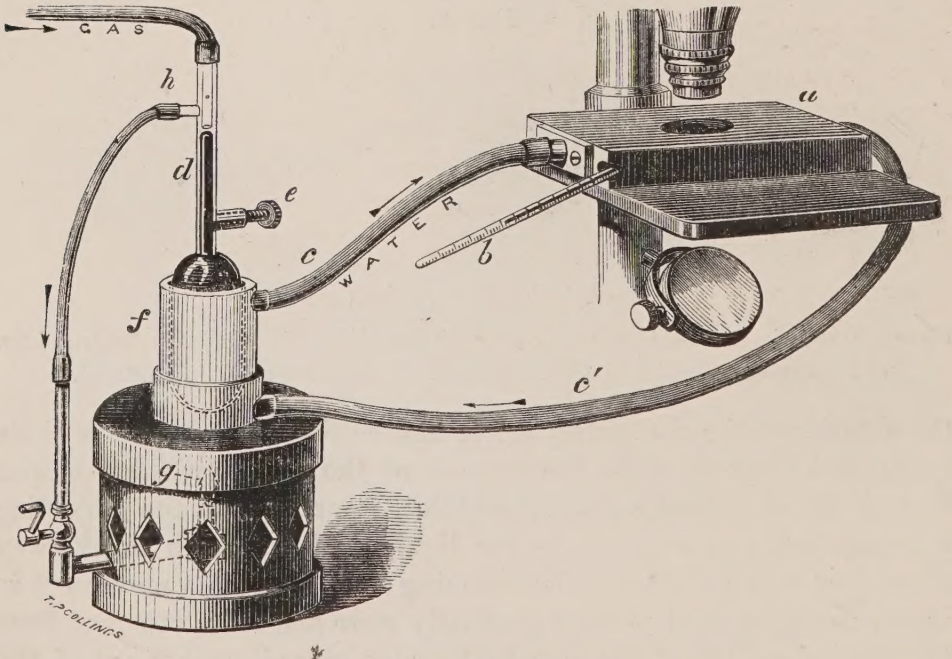
the exterior of the clump (fig. 8, *b*); and as the filament gets a little longer and projects more the remains of the disk may often be seen forming an enlargement near its middle. Soon some of the projecting filaments begin to oscillate in the liquid, and at length break away altogether from the mass, and continuing their oscillatory movement in the surrounding fluid, become gradually more and more removed from the clump, until they may eventually even pass altogether out of the field of the microscope. Meanwhile other of the disks have grown out into filaments and taken the place of the liberated superficial ones, and soon these in their turn become free and give place to others; so that in this way the previous solid-looking mass becomes almost entirely broken up into freely-moving filamentous particles (fig. 8, *c*). These are not all of the shape above described, but present various forms, as shown in the accompanying figure.

The above observation shows that the discoid particles which

are occasionally found in the blood, and which when the blood is drawn tend to become collected into clumps, are to be regarded as centres of origin from which minute, filamentous, bacterium-like organisms may under some circumstances be developed; but whether this change ever occurs in the living body, and what further modification the filaments may, under conditions more favourable for their development, undergo, is at present undetermined.

After the observations recorded in the preceding paragraph are completed the action of an excess of heat may be observed; but it is better to use for this purpose a larger apparatus, in which the degree of heat can be measured by a thermometer. Such a one is shown in fig. 9. In this the

Fig. 9.



Apparatus for maintaining a constant temperature under the microscope.

preparation is placed upon the brass box, *a*, which rests on the stage of the microscope, and is pierced in the centre by a tubular aperture, to admit light to the object. The box is connected by india-rubber tubes with a hollow metal jacket, *f*, and the whole system thus constituted is completely filled with water previously boiled, to the exclusion of air. The

water is warmed at *g* by a small gas-flame, and rising through the tube *c* communicates its heat to the box *a*, the temperature of which is measured by a small thermometer *b*, inserted through an obliquely placed tube, quite into the central hole and immediately under the preparation. The cooled water from the stage descends down the tube *c'*, to pass again round to the flame, and in this way the water constantly circulates. The bulbed tube *d*, filled with mercury, serves to regulate the flow of gas, so as to keep the temperature constant at any desired point. This is effected by turning the steel screw *e*, when this point, whatever it may be, is reached, so as to raise the mercury in the glass tube, and thus almost block up the lower end of a small steel or glass tube, which is fixed into the upper end of the tube *d*. The gas passes through the small tube, and then above the mercury and between the two tubes to be conducted by the side piece *h* to the burner below, and it will be understood that if the temperature now rose higher in the reservoir *f*, which surrounds the mercury, this on being warmed will expand and tend to cut off more of the gas and thus reduce the flame, on which the mercury will again contract, and the flame will rise in consequence, and so on. It is found that an equilibrium soon becomes established, and the temperature of the water and stage remains almost absolutely constant. To raise or lower the temperature all that is required is to screw out or in the screw *e*. The small included tube is pierced with a minute aperture, to allow a constant passage of gas, so as to prevent the flame from being extinguished in the event of the complete occlusion by the mercury of the lower end of the tube in question.

By employing this or a similar apparatus it will be found that up to and a little beyond the normal temperature of the blood the white corpuscles become more active in their movements, but on gradually warming the preparation still more a point is soon reached at which they draw in their processes, become spherical, and show no longer any signs of vitality—the temperature being sufficient to kill the corpuscles. The

red corpuscles remain unaltered until a temperature of about 55° C. is reached, when they become altered in shape, and globular; eventually their colouring matter is discharged and becomes dissolved out in the surrounding serum.

Action of Reagents upon the Blood.—The red corpuscles in the two preceding preparations appeared, even under the highest power, perfectly homogeneous and structureless, but it can be shown, by the application of reagents to the blood under the microscope, that they in reality consist of two intimately mingled but separable parts—the *stroma* (formed of various chemical compounds, such as cholesterin, paraglobulin, &c.), which is colourless, and gives the shape to the whole corpuscle; and the *coloured part*, which consists chiefly or wholly of a red crystallizable substance, hæmoglobin. The mode of application of reagents is as follows:—A drop of blood is got ready as in the first preparation, and whilst under observation a small drop of the reagent (which should as a rule be freshly prepared) is allowed to come into contact with the edge of the cover-glass. Some of the fluid flows under this and mixes with the drop of blood; the current produced by it at first drives the corpuscles before it, but they soon become stationary, and then the part of the preparation should be selected for observation where the reagent is gradually diffusing itself amongst the corpuscles. In this way every stage in its action may readily be studied.

Preparation 3. Action of water.—When a drop of distilled water is applied in the manner above described the first effect is seen to be that the red corpuscles begin to lose their discoid form, first one of their sides becoming bulged out, so that they are cup-shaped, and then the other side, so that they are now completely globular, as may be seen when they roll over. Meanwhile the hæmoglobin is being dissolved out of the corpuscles by the water, so that they are soon quite colourless and hardly to be detected in the now reddish fluid. Some seem to offer greater resistance to the action of the water (and indeed of most reagents) and to retain their colouring matter longer than others.

The white corpuscles are also soon affected. They cease their amœboid movements and begin to swell up by imbibition of fluid, whilst at the same time with a high power the granules in their interior may be seen to exhibit the dancing movement which is characteristic of minute particles floating in liquid. Often the corpuscles present clear bulgings at their circumference, or their substance may appear to burst at one point and become diffused in the water. As they swell and become clearer, one, two, or more small round nuclei generally come into view, and soon these also become swollen, and with the rest of the corpuscles eventually disintegrate, nothing being left but a few granules.

Water is thus proved to have a characteristic action upon the protoplasmic white corpuscles as well as upon the very easily alterable red disks, and this fact must be borne in mind in investigating the action of reagents or poisonous substances, both upon the blood-corpuscles and upon the tissues generally. If a reagent is to be employed in weak solution, therefore, it is well to dissolve it either in salt solution or in fresh serum [1] instead of water. Washing with water tissues which are subsequently to be submitted to microscopical examination, as is so freely done in the post-mortem room, is for a similar reason to be deprecated; but if a trace of bichromate of potash or of chromic acid, or a little common salt be previously added to the water, its deleterious effect upon the protoplasm is in great measure obviated.

Preparation 4. Action of Acetic Acid.—To investigate the action of dilute acids it is best, as just explained, to mix the acid with salt solution instead of water: 1 part of glacial acetic acid to 200 of salt solution is an appropriate strength for the blood. The preparation is made in the usual way, and the drop allowed to run in at the edge of the cover-glass. The action of the weak acid upon the red corpuscles is seen to be quite like that of water: they

[1] The serum employed must be from the blood either of the same animal or of one belonging to the same species.

are first rendered globular and then decolorised. Upon the white corpuscles it has a somewhat different action, for although the protoplasm of the corpuscle becomes partly swollen out into a clear spheroid, the nuclei are not swollen by the reagent, but are brought very distinctly into view, as well as the nucleoli within them, and remain usually at one side of the corpuscle, with a little granular matter precipitated around them.

Preparation 5. Action of Tannic Acid.—The action of tannic acid upon the red corpuscles is peculiar and interesting. Like other acids it tends to cause the coloured part of the red corpuscle to become separated from the stroma; but as the coloured material is exuding it becomes coagulated by the astringent reagent, and in place of being dissolved in the surrounding liquid, as after the action of acetic acid, it remains attached to the stroma as a small, bright, reddish projection.

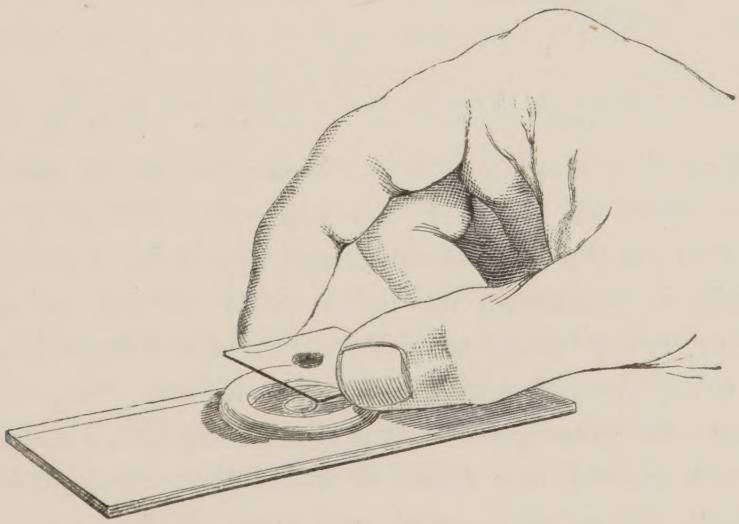
In the first part of the reaction—viz. the rendering the corpuscles globular—it acts similarly to other weak acids.

The most convenient strength of solution to use is 1 per cent.; it should be freshly prepared. It is well, moreover, first to mix the drop of blood upon which it is desired to test the action of the reagent with an equal amount of salt solution; otherwise the tannic acid produces such a dense precipitate with the albumin of the serum that the view of the corpuscles is greatly obscured, and indeed the reagent with difficulty reaches them.

Preparation 6. Action of Weak Alkalies.—A mixture of 1 part of caustic potash to 500 of salt solution may be used, a drop being added to the preparation in the usual way (p. 24). The reaction takes place very suddenly; the corpuscles, both white and red, swell up as soon as the reagent reaches them, appear to burst, and then entirely disappear. The white are affected by a weaker solution than the red. This is apparent from the fact that, as the liquid slowly diffuses and mixes with the blood, the white may be seen to become destroyed in parts where the red are still unaffected.

Preparation 7. Chloroform.—To observe the action of chloroform vapour upon blood a moist chamber is used. This is an apparatus for keeping a tissue or fluid under examination in its naturally moist condition, whilst at the same time allowing its surface to be exposed to air or to any desired gas or vapour. The simplest form of moist chamber is made out of a small piece of soft putty or modelling-wax, which has been rolled out between the fingers into a round cord about 2 inches long and $\frac{1}{8}$ inch thick; the ends of the cord are united so as to form a ring, and this is placed on the middle of a clean glass slide. A drop of water is put into the centre of the ring; this is for the sake of keeping the atmosphere of

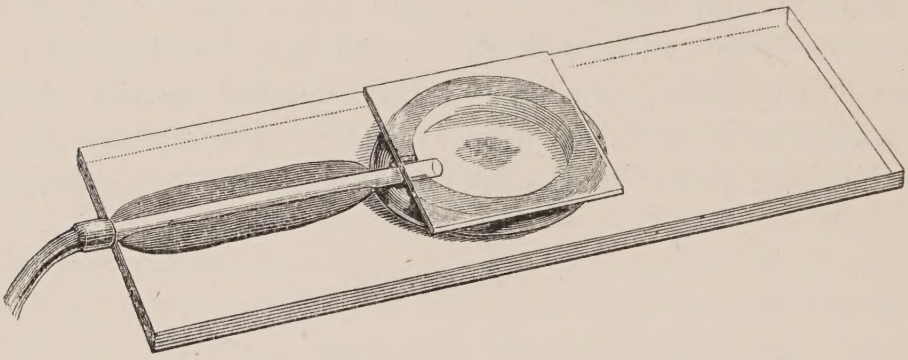
FIG. 10.



the chamber moist; but the object may be equally well effected by breathing into the space as it is being covered over. The object is prepared on the centre of a clean cover-glass, which is then inverted over the ring, so that the preparation is dependent into the chamber, and, whilst freely exposed to the air in this, is entirely protected from evaporation and may be readily examined through the cover-glass, to the under surface of which it remains adherent (fig. 10).

To investigate the action of chloroform vapour it is necessary to have some means of passing this into the moist chamber, whilst the drop of blood is under observation. For this purpose a slide is employed (fig. 11), to which a piece of small glass tubing has previously been fixed by means of sealingwax, and the ring of putty is so placed as to include

Fig. 11.



Chamber for passing a gas or vapour over a preparation under the microscope.

the end of this, a small interval being left at the side of the tube to afford an exit for the vapour. The slide is then clamped on to the microscope stage (as in fig. 13 *s*), and the glass tube is connected by indiarubber tubing to a bottle containing a few drops of chloroform and furnished with a second tube, through which air can be blown.

Before the cover-glass is superposed the blood should first have been spread out upon it into a thin layer, so that the chloroform vapour may readily act upon all parts.

Everything being thus prepared, and some of the blood-corpuscles having been brought clearly under observation, air is blown gently into the bottle, and passing through it becomes charged with the vapour of chloroform, which is conveyed by the tube into the moist chamber, where it acts upon the layer of blood which is on the under surface of the cover-glass. After a short time it will be seen that the red corpuscles, as under the action of water and dilute acids, at first become globular, and subsequently their hæmoglobin becomes

dissolved and discharged out in the serum. It will further be observed, both in this and in the other preparations in which this change has taken place in the red corpuscles, that to the naked eye the blood has changed from scarlet to lake, and that whereas when the corpuscles were intact even a thin layer of blood presented a somewhat opaque appearance, it is now completely transparent. Hence we may infer that the opacity of the unaltered blood is due to the presence of the red particles.

Preparation 8. Examination of Frog's Blood.—The simplest way to obtain frog's blood for examination is to cut off the tip of one of the digits, having previously wiped it dry with a cloth, and to collect upon a clean cover-glass the small drop of reddish fluid which exudes. The glass is then inverted upon a slide and the drop is examined. The blood so obtained is mixed with lymph, and the corpuscles are consequently less crowded and better adapted for observation than when the blood is undiluted. To procure blood unmixed with lymph the animal should be pithed, and laid upon its back. The heart is then exposed and snipped with scissors, and a small drop of the blood which exudes is taken up with a glass rod, transferred to a slide and covered. But before placing the cover-glass down in the usual way a small length ($\frac{1}{4}$ inch) of a delicate hair should be placed in the drop, so that when the cover-glass settles down, the corpuscles, here comparatively large, may not be crushed by its weight.

When the preparation obtained from either of these sources is examined it will at once be seen that the coloured corpuscles are larger and fewer in number than in human blood, and do not tend to form rouleaux; that they have an elliptical outline when lying flat, but when seen edgeways look quite narrow and pointed at the ends, with a slight and gradual bulging at either side; so that, although disc-shaped, like the mammalian blood-corpuscle, so far from being biconcave, they are biconvex. The bulging is due to the

presence in the middle of the corpuscle of another part besides the stroma and the coloured substance found in the mammalian disc. This, the so-called nucleus, can readily be made out in most of the corpuscles as a somewhat elongated, colourless, and slightly granular elliptical body, about a third the length of the corpuscle, and often lying not quite in the middle but somewhat excentrically. Occasionally the nucleus is seen to be round; but this is an accidental change, and may be brought about by mechanical injury. Indeed, if the precautions above recommended for avoiding pressure are not taken, a large number of the corpuscles become injured, so much so as even to be ruptured and destroyed altogether, in which case the rounded nuclei are liberated, and may be mistaken by the beginner for pale blood-corpuscles.

With regard to the white corpuscles, it will be observed that they are more numerous and larger than in human blood. Moreover, they soon begin, even in the cold, to exhibit very distinct amœboid movements; and, on account of the greater size of the corpuscles, both these and the other phenomena exhibited by them are much more striking, and these corpuscles are therefore much better suited for observation than those of mammals, which in other respects they closely resemble. The distinction between the finely- and the coarsely-granular corpuscle is met with again here; but there is also sometimes seen a third kind of corpuscle, fusiform or oat-shaped and devoid of amœboid properties. It has been suggested that these are cells which have belonged to and have become detached from the lining membrane of the blood-vessels.

Preparation 9. Feeding of the White Corpuscles.—The white corpuscles of the blood exhibit a strong tendency to take into their interior any small particles of insoluble materials which may happen to be in their neighbourhood. This tendency they have in common with all amœboid organisms, and it is nowhere better seen than in the case of the amœba itself. Because of their greater size, number, and activity, it is better

to take the frog's corpuscles for this experiment rather than those of mammalia.

To obtain the white corpuscles in quantity, and comparatively free from coloured ones, take a capillary tube (like those used for preserving vaccine lymph in, which can be easily made by heating a piece of glass tube red-hot in the blowpipe-flame, and then removing and drawing it out before it has time to cool), and allow it to fill with blood from the heart, exposed as above directed. Now lay the tube aside for an hour, and meanwhile the particles with which the white corpuscles are to be fed may be prepared. This is done in the following way:—A few drops of salt solution are poured into a watch-glass, and a cake of Indian ink is rubbed in it until when thoroughly mixed up the fluid has acquired a faint greyish colour. When left to itself for a time the coarser particles subside, whereas the finer ones remain longer suspended. At the expiration of the hour the blood is taken and its contents are blown out upon a glass slide, the clot which has formed within the tube being removed, leaving merely the exuded serum. This contains a few red blood-corpuscles and a large number of white ones, the latter having by virtue of their amœboid movements migrated, during the space of time the tube has been left, from the clot into the serum. With the drop of serum a little of the salt solution containing fine suspended particles of the pigment is thoroughly mixed, and the preparation is then covered, the edges of the cover-glass being surrounded with oil or melted paraffin, to prevent evaporation. When the preparation is observed it will be seen that the white corpuscles have quite their normal appearance, whereas the coloured ones for the most part exhibit folds or plaits upon their surface, having undergone a slight shrinking, owing to the increased density of the fluid in which they lie. The black particles from the Indian ink are scattered irregularly over the field; and although they may here and there be in contact with the white corpuscles, it is nevertheless to

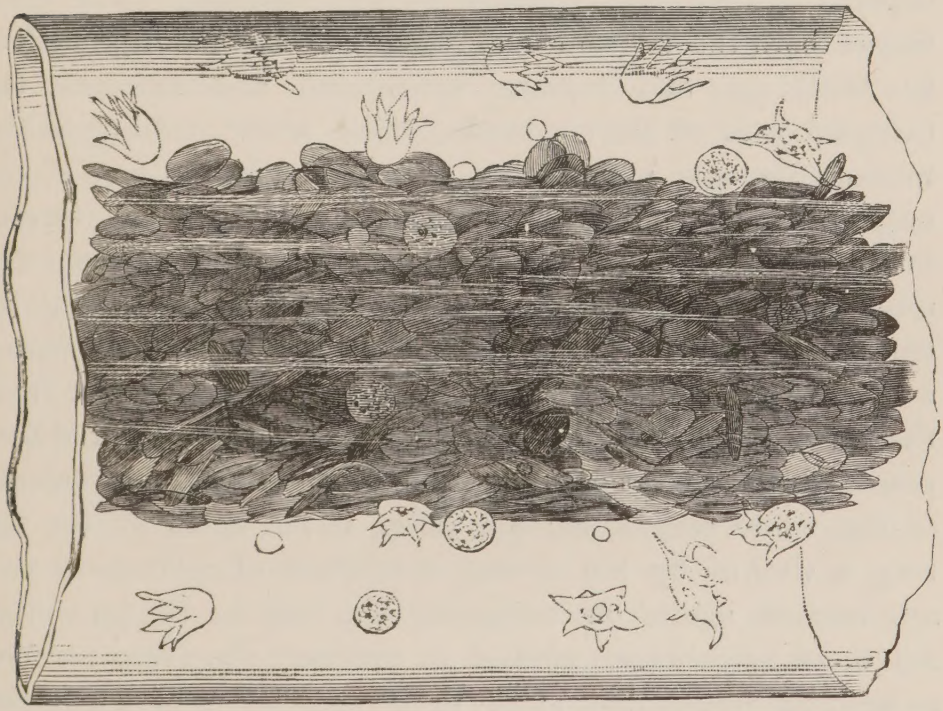
be readily made out that the substance of the latter is entirely free from pigment. But after a short time, if the corpuscles are closely observed, it will be seen that they are gradually taking into their substance the black particles which were in contact with them; and when by their amœboid movements from one place to another they are brought into contact with other particles these also are in like manner absorbed. On careful watching it may be made out that the process of intussusception commences by the throwing out of processes which surround the particle to be taken in, and meet and coalesce beyond it; once included in this manner, the granule afterwards becomes gradually carried, presumably by the movements of the protoplasm, more towards the centre. If, after observing the preparation in this way for half an hour, it be laid aside for two or three hours, it will be found at the expiration of that time that many of the white corpuscles have taken in a large number of the black particles, for they do not discharge their cargo, but carry it about in their movements from place to place.

This property of the white blood-corpuscles has been used in pathological investigations to determine the source of the corpuscles of pus, which resemble the white corpuscles of the blood. Insoluble particles were injected into the blood-vessels of an animal, and inflammation was induced at a special part: it was found that the pus-corpuscles which accumulated outside the blood-vessels in the inflamed part contained similar particles. The corpuscles must consequently have come from the blood, for such particles have not the power of passing through the coats of the blood-vessels unless carried by the white corpuscles.

Preparation 10. Migration of White Corpuscles.—The process of migration can be quite easily seen in progress in transparent parts of animals, and will be afterwards studied when the methods of observing the circulation of the blood are described. But for exhibiting the active migration of the white blood-corpuscles nothing is more striking than the examination of a capillary tube in which frog's blood has

been collected and has coagulated. A high power is to be employed, and the capillary tube must therefore be very fine indeed; and in order that the wall of the tube should be as thin as possible, it must be drawn out from a piece of glass tubing half an inch or more in diameter. The capillary tube is filled with frog's blood, except near the ends; these are then sealed by holding them successively in the flame for a second or two; the tube is then placed in a drop of glycerine on a slide, covered with a thin glass, and at once examined. The object of the glycerine is to correct in some measure by

FIG. 12.



White corpuscles of frog's blood migrating from clot. Highly magnified. The clot has shrunk considerably from the sides of the capillary tube.

its high refracting power the effect upon the light of the cylindrical glass tube. After a few minutes the clot is seen to be getting smaller, and a layer of clear serum collects between it and the glass; the quantity of this gradually increases, and soon one or two white corpuscles begin to project from the surface of the clot. These protrude more and more,

and others make their appearance, and all begin to throw out numerous amœboid processes, which are actively advanced and retracted, and by aid of which the corpuscles free themselves entirely from the shrinking clot, and eventually become free in the surrounding serum (fig. 12).

Preparations 11, 12, 13, 14. Action of Reagents—Water, Acetic Acid, &c.—upon the Frog's blood-corpuscles.—The action of reagents upon the white blood-corpuscle of the frog is exactly the same as upon the mammalian white corpuscle; upon the red corpuscle it is in the main similar, but in some cases a little different, the differences partly depending upon the presence of the nucleus. Thus *water* causes both the substance of the corpuscle and the nucleus to swell up and become spheroidal, and extracts the coloured part of the corpuscle; dilute *acetic acid* brings the nucleus strongly into view, and decolourises the rest of the corpuscle, which, however, usually retains its oval shape; *tannic acid* causes the coloured part to be exuded from the stroma, to which it generally remains attached as an irregular curdled mass (sometimes the coloured part is precipitated around the nucleus, and then the two may be ejected from the stroma together); *chloroform* vapour causes the red corpuscles to become decolourised, and arrests the movements of the white corpuscles; but these, if not acted on for too long a time or by too strong a mixture of chloroform and air, resume again on replacing the vapour by pure air. All these reagents are applied in precisely the same manner as with the mammalian blood, to the description of which the reader is referred.

The observations may be made with as great or with greater advantage upon the blood-corpuscles of the newt, which are larger than those of the frog. The blood should be obtained directly from the heart, and not by merely snipping a piece off the tail and collecting the drop which exudes, for in this case it is very apt to become mixed with the acrid secretion from the cutaneous glands. There are

in addition two reactions for which newt's blood is particularly well adapted, viz., the actions respectively of boracic acid and carbonic acid upon the red corpuscles.

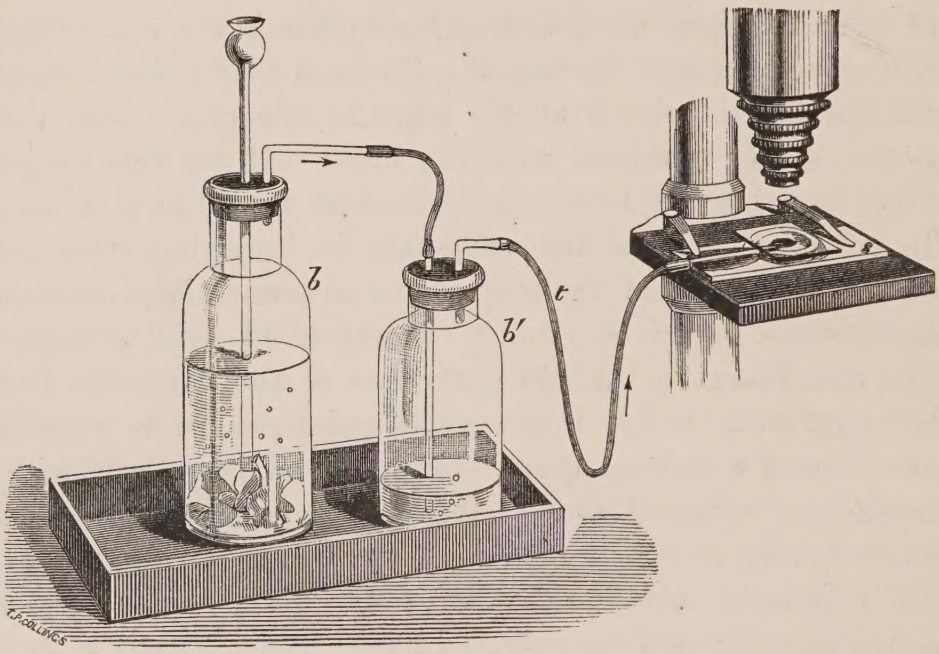
Preparation 15. Action of Boracic Acid.—The boracic acid is used in solution in water (two parts per cent.), and the preparation of blood having been made in the usual way, with or without addition of salt solution, a drop of the boracic acid solution is placed at the edge of the cover-glass and allowed slowly to mingle with the blood. If the first stages of the reaction are fortunately observed it will be seen that the coloured part of the corpuscle is becoming collected towards the centre of the corpuscle and accumulated around the nucleus, often remaining, however, at first adherent here and there to the circumference of the corpuscle, and shrinking away at the intermediate points, so as to present somewhat of a stellate figure.[1] But soon it is entirely withdrawn and collected around the nucleus, which has become rounded, and is nearly concealed by the colouring matter. The corpuscle, now decolourised, has also in many cases become circular, and the coloured nucleus is generally shifted to one side, and eventually altogether extruded.

Preparation 16.—To investigate the action of carbonic acid gas the blood must be prepared in a moist chamber, like that used for chloroform vapour (fig. 11). If the preparation is very quickly made the nucleus in many of the red corpuscles cannot at first be distinguished, for in the entirely unaltered state it possesses as nearly as possible the same index of refraction as the rest of the corpuscle. But when carbonic acid gas, generated in a suitable apparatus (fig. 13, *b*, *b'*), is allowed to pass into the moist chamber, a precipitate of one of the constituents of the stroma (probably paraglobulin) occurs around the nucleus, and the outline of this is brought into view, whereas if the carbonic acid is speedily replaced by air, which may be effected by disconnect-

[1] Water and various other reagents may, in the first instance, have a somewhat similar effect.

ing the tube *t* from the wash-bottle *b'* and drawing air through it by the mouth, the precipitate is re-dissolved, and the nucleus is again made to disappear.

FIG. 13.



Apparatus for passing carbonic acid gas over a preparation under the microscope.

b, bottle containing marble and hydrochloric acid ; *b'*, wash-bottle ; *t*, india-rubber tube conducting the gas to the stage, *s*.

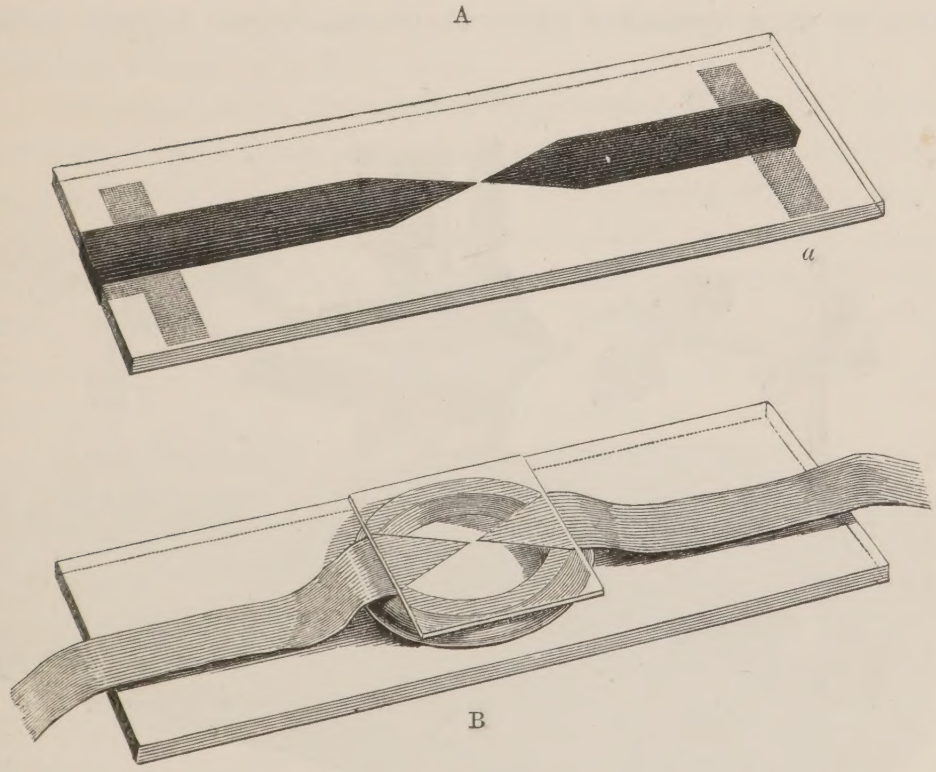
It is of advantage in performing this experiment to add a trace of moisture to the blood before the observation; this may be most conveniently done by breathing two or three times on the preparation before placing it over the ring of putty.

Preparation 17. Influence of warmth on white corpuscles of Amphibia.—The action of gentle warmth in accelerating the movements of the pale corpuscles of the frog or newt may be investigated with the same apparatus as was used for the observation of mammalian blood at the temperature of the body. But it will be found that if the temperature be allowed to rise s high as 38° C. the movements of the corpus-

cles of these cold-blooded animals will soon permanently cease, the corpuscles being killed. So that unless this result is desired, some fat of lower melting-point must be employed to indicate the temperature limit which is not to be exceeded.

Preparation 18. Action of Electric Shocks.—For this a slide must be specially prepared by cementing on to its upper surface with shellac varnish two slips of gold-leaf or tin-foil,

FIG. 14.

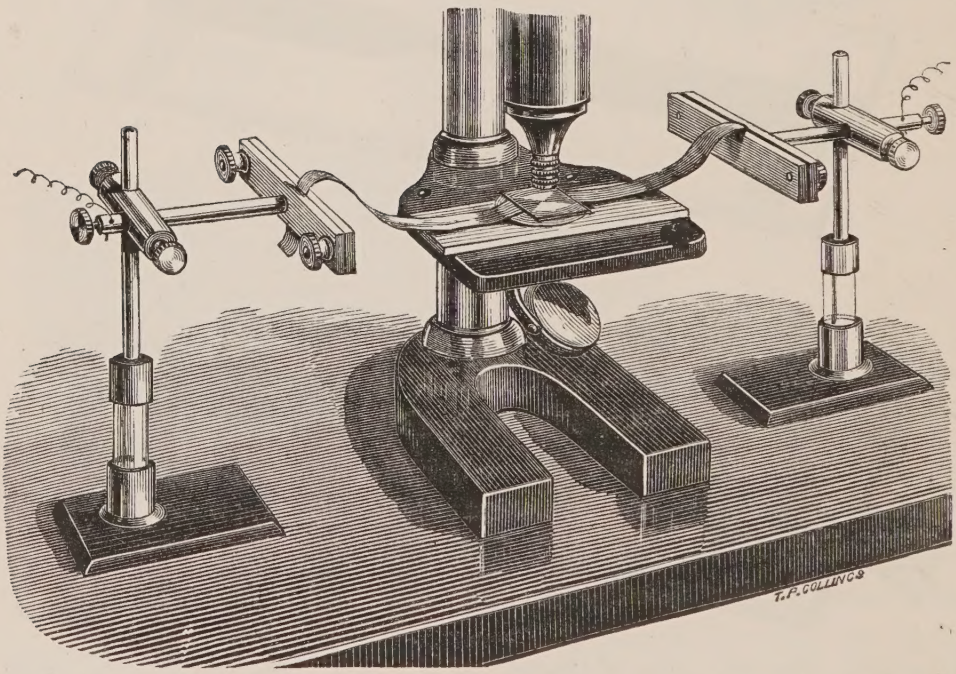


Glass slide, with two strips of tin-foil, one of which passes round to the under surface, where it rests on the brass stage of the microscope ; the other strip is isolated from the stage, and may be connected to the outer coating of a Leyden jar, the charge of which is made to pass between the points by connecting the knob of the jar with the brass work of the microscope. Opposite *a*, a small piece of the foil is fixed to the under surface of the slide, so that this end shall be level with the other.

with pointed ends which almost meet in the middle of the slide (fig. 14, A). A drop of blood is put here, the cover-glass is placed over it, and the portion of blood which lies between

the points is brought under observation. Or a moist chamber may be employed, the cover-glass used having previously had two strips of tin-foil cemented to it (fig. 14, B). The drop of blood being spread out in a thin layer between their points, is quickly inverted over the ring of putty and brought under observation. The tinfoil slips are kept isolated from the brass-work of the microscope, and are so arranged that the charge of a small Leyden jar, or an induced current of electricity, can be passed through them at any moment (fig. 15). One or more amœboid white corpuscles which happen to be

FIG. 15.



Apparatus for passing electric shocks through a drop of blood, which is to be examined in a moist chamber. The tin-foil slips are cemented near their points to the under surface of the cover-glass, and their free ends are clamped to isolated metal supports, connected by wires to an induction-coil. The tin-foil slips are isolated from the brass stage of the microscope by the glass slide on which they rest.

in the path which the spark must take in traversing the interval between the points are kept in view, and the spark is then allowed to pass. The white corpuscles immediately

cease moving, withdraw their processes, and become rounded in shape; in fact, undergo general contraction. But if only one slight shock be given they soon recover and resume their movements, although these are often somewhat altered in character. The red corpuscles are but slightly if at all affected; but if a succession of shocks be transmitted from an electric machine or an induction-coil, electrolytic action is set up in the fluid, bubbles of gas are developed, the effects respectively of acids and alkalies are set up in the neighbourhood of the tin-foil points, and the red corpuscles undergo changes brought about by these.

Preparation 19. Presence of Glycogen.—Many of the white corpuscles contain a certain amount of glycogen, or animal starch, either in distinct granules or in a more diffused form. This substance becomes stained of a reddish mahogany colour by solution of iodine, and may thus be readily detected, both here and elsewhere. The solution to be used is made by dissolving 1 gramme of iodine in 100 cc. of water, which contains 2 grammes of iodide of potassium in solution.

The preparation, preferably of frog's or newt's blood, is made in the usual way, and the iodine solution added at the side of the cover-glass. The red corpuscles are stained of an intense yellow, but are otherwise little altered, except that the nucleus, which remains unstained, becomes globular, and bulges out at either surface of the corpuscle. The white corpuscles are instantly arrested in their movements and killed, preserving exactly the form which they exhibited when reached by the iodine solution. Being of less specific gravity than the latter, they tend to float on it; and, if the layer of fluid is thick, must be sought by focussing upwards in the stratum immediately under the cover-glass. The main substance of the corpuscle is uniformly stained of a deep yellow, but many contain groups of mahogany-stained granules, and from others are seen to exude after a time pellucid drops of varying size, which become tinted of a mahogany or port wine colour, and no doubt contain glycogen.

Preparation 20. Crystals obtainable from the Colouring Matter of Blood.—The hæmoglobin or colouring matter of the blood may be obtained in definite crystals, but the form of the crystals varies in different animals. It is difficult to induce the crystallisation in human blood; and to obtain the crystals readily it is best to employ the blood of the rat.

A drop of this is mixed with an equal amount of distilled water, covered, and observed under the microscope. The water has the effect of extracting the hæmoglobin from the corpuscles; until this is done no crystallisation takes place. As the excess of water begins to evaporate at the edges of the cover-glass small needle-shaped crystals of hæmoglobin begin to appear, either singly or in bunches, which become larger and larger until they may attain a very considerable size.

Preparation 21.—Crystals may also be readily obtained from guinea-pig's blood, either by the same method or more readily perhaps in the following way:—

The animal is decapitated, and the blood as it flows from the divided vessels is vigorously stirred with a small bundle of wire, to remove the fibrin. A small quantity of the whipped blood is then mixed with about one-third its volume of water, and a drop of chloroform being added, the mixture is thoroughly shaken up for a minute or two. This has the effect of discharging the hæmoglobin from the corpuscles into the surrounding fluid. A small drop is now placed upon a slide and left exposed to the air for a few minutes. It becomes thickened by evaporation and dried at the edges, and crystals, tetrahedral in form, may be detected in it with a low power of the microscope. The preparation may then be covered and examined with a higher power. The crystals increase in size for a time, and new ones continue to be formed.

Hæmoglobin crystals will not keep unaltered for any length of time.

Preparation 22.—The name 'hæmin' has been given to certain very characteristic crystals which are formed at the

expense of the colouring matter of the blood, and the production of which serves as an exceedingly trustworthy test of the presence of blood, although yielding no indication of the kind of animal from which the blood has been obtained. To see them a very small quantity of blood obtained from the finger or elsewhere is smeared upon a glass slide and allowed to dry. A cover-glass is then placed over it, and a drop of glacial acetic acid is applied from a pipette to the edge of the cover-glass and allowed to run under by capillary attraction. The slide is then held by one end in the fingers, and the middle is gently warmed over a small flame. As soon as bubbles begin to appear in the fluid the warmth is discontinued, and the preparation is examined with a high power. If no crystals appear as the slide cools a little more acid is added, to replace that lost by evaporation, and the slide is warmed as before, and on cooling again examined. It will be found that almost all over the preparation reddish-brown short prismatic crystals, disposed singly or in groups, will have made their appearance; most of them are very minute, but they may be obtained of considerable size by re-warming the preparation with glacial acetic acid once or twice.

The presence of a chloride is necessary for the formation of these crystals; in the case of recent blood the chloride of sodium which it naturally contains is sufficient for the purpose; but if it were an old blood-stain which one had to deal with, in which the chlorides may have been washed away, it would be previously requisite to mix a minute quantity of common salt with the stain to be tested in order to supply the deficiency.

CHAPTER II.

THE EPITHELIAL TISSUES.

THE epithelial tissues are studied with regard both to the character and form of the individual elements, and the relations these bear to one another and to the membranes they cover. The latter class of observation can only be properly made by the study of sections of the various organs and parts where epithelium is found, and will therefore be left until the method of making these is explained. The modes of isolating and studying the individual cells will, however, be best described in this place.

Preparation 1. Scaly Epithelium. Superficial Layers.— If a little of the saliva which moistens the inside of the cheek be gently scraped off with a small spatula or with the finger-nail, a number of the superficial cells of the thick stratified epithelium which is here met with will be brought away with it. The material thus obtained is placed upon a slide and a cover-glass put over. On examining the preparation numerous flattened scaly epithelium cells will be seen, either entirely isolated or in little patches, the cells in a patch being connected together, with their edges overlapping. The cells are of considerable size, each with a nucleus near its middle, small in comparison with the size of the cell; and the substance of the cell, although clear, yet contains a number of scattered granules. Moreover, lines may often be seen running in various directions over the surface; these are for the most part due to folds or creases of its substance. Some of the cells may be seen edgeways, and then, being flattened, will appear narrow and linear; but on touching the cover-glass with a bristle their true form will be apparent as they turn over.

In addition to such cells as these a certain number of much smaller rounded cells may generally be seen in the saliva, which somewhat resemble the white corpuscles of the blood, and, like them, frequently exhibit amœboid movements. They are, in fact, to be regarded as white blood-corpuscles which have either migrated from the blood-vessels into the salivary ducts and have been carried into the mouth with the saliva, or have come from the mucous membrane covering the back of the tongue and the tonsils, which is very rich in lymph-corpuscles. The saliva being a watery fluid they are swollen out by it, and with a good microscope it may be observed that the granules in the interior of the corpuscles exhibit the Brownian molecular movement, a phenomenon which, it will be remembered, was exhibited by the white corpuscles of the blood, as a first result of the imbibition of water.

Preparation 2. Deeper Layers.—There are several layers of the above described large flattened epithelial cells in the epithelium of the mouth. Below them are other cells smaller and of a more spheroidal or polyhedral shape, and many of them having ridges and spines with intervening furrows upon their surface. To obtain these isolated it is necessary to macerate a piece of any membrane which is covered by a similar scaly stratified epithelium in some fluid which, while it softens and dissolves the intermediate substance which cements the cells together, may preserve their natural form, and, at the same time, prevent putrefaction from appearing in the tissue which is undergoing maceration. The best liquid for this purpose is a weak solution of bichromate of potash, 1 part to 800 of water ($\frac{1}{8}$ per cent.). The portion of tissue must be small, and the quantity of liquid used comparatively large. A piece of the mucous membrane of the mouth, pharynx, or gullet of any mammal may be used; it will require at least two or three weeks' maceration (for in this kind of epithelium the cells are very closely united), and the bichromate solution should

be changed every third day. At the expiration of the time stated a small portion of the epithelium is scraped off with the point of a knife and placed in a drop of water upon a slide. It is then broken up as finely as possible with a pair of mounted needles, a piece of a hair is cut off and placed in the drop, and the cover-glass is superposed. By far the majority of the cells seen are the superficial ones already described; but others may be found which are less flattened and smaller in diameter, and have many of them an irregular toothed margin, their surface also, as may be seen by altering the fine adjustment of the microscope, having on it linear or punctated markings. These are the cells with ridges and spines above mentioned; they will be again studied in sections of the skin, as well as in sections of these and other mucous membranes which are covered with similar epithelium.

Preparation 3. Horny Layer of Epidermis.—If a very small shred of the superficial part of the epidermis is taken from any part, the palm of the hand, for instance, and examined in water under the microscope, no indications of cellular structure are visible—nothing but a hard horny confused mass is to be seen. Remove the cover-glass and place the shred of epidermis in a drop of liquor potassæ. It will soon swell up and become soft. When this is the case return it to the water, and, after breaking it up as finely as possible with needles, cover and examine. Numerous spheroidal cells are now seen loose in the preparation, with a distinct contour, as if enclosed by a membrane, but without a nucleus. They are, in fact, the scaly cells, which have become swollen out by imbibition of water, and at the same time, in consequence of this swelling of their surfaces, correspondingly diminished in width.

The same result may be obtained with the cells which form the nails; and also with the flattened cells from the mucous membrane of the mouth.

Preparations 4, 5, 6.—The Columnar Epithelium is most characteristically seen and is best studied as met with in the

intestinal canal. It must be taken from an animal (rabbit) quite recently killed, as it rapidly undergoes destructive changes if left after death in contact with the intestinal contents. When the intestine is cut across at any part in a recently killed animal the cut edges curl outwards, and a little of the mucous membrane is thus exposed. Two very small portions may be snipped off this and placed, one in a few drops of a one per cent. solution of osmic acid, and a second in iodized serum.[1]

These two portions may be put aside for the present in their respective fluids, whilst a preparation of the epithelium in the fresh condition is made and examined. For this purpose slit open a piece of the intestine, wash away the mucus and intestinal contents by allowing a little of the serum from the animal's blood to drop upon the inner surface, and then, with the end of a clean scalpel, gently scrape the washed surface, and transfer what is brought away on the scalpel to a drop of fresh serum upon a clean glass slide, and cover the preparation, averting the pressure of the cover-glass by means of a piece of hair. On examining with a high power the specimen so obtained numerous columnar epithelium cells will be seen, some separate, others in groups. (It will be found advantageous in examining the object to moderate the illumination by the employment of one of the smaller holes of the diaphragm: in this way the somewhat indistinct outlines of the tissue-elements are rendered plainer, and any details of structure can generally be more readily made out; this is the case,

[1] This is prepared from the amniotic fluid of the cow, by adding to it a crystal or two of iodine, and allowing it to stand for a few days, with frequent agitation, and then filtering off the clear fluid from any precipitate that may have formed. Almost any other fresh serous fluid can be used instead, but the amniotic fluid is generally the most readily obtained in sufficient quantity, and at the same time perfectly clear and pellucid. The serous fluid serves to macerate tissues which are immersed in it, whilst preserving as nearly as possible the original form of their elements. The purpose of the iodine is to prevent putrefaction; it serves also at the same time to render the tissue-elements slightly firmer.

indeed, with most preparations.) In the separated cells, the conical form may be seen, the cell either tapering at one end to a fine point, which may be bifid, or terminating in a rounded extremity. The general substance of the cell has a faintly granular appearance in the fresh condition, without a distinct outline, except at the larger end, which is bounded by a strongly refracting thickened margin, in which a few faint striæ passing from without inwards may with a high magnifying power be made out. Near the centre of the cell is the clear oval nucleus, bounded by a distinct line, and containing generally one nucleolus. Globules of fat of varying size may, if the animal were killed during digestion, be seen within the cell; they are recognised by their strong refractive power.

Of the groups of cells some may occur in which the cells are seen from above; the collective bases will then present an appearance of polygonal areas intersected by lines of intercellular substance; in other groups, where the cells are seen laterally, their arrangement with regard to one another will be observed.

Some cells will probably be seen which have acquired a peculiar chalice-like shape, owing to the part of the cell near the free surface having become swollen out, often to the extent of bursting away and destroying the thickened border. These are the so-called 'goblet cells.'

The other two portions of tissue will not be ready for examination for some days. That in the iodized serum must be put aside in a cold place, in a well-stoppered bottle. At the expiration of a week a preparation may be made from it by scraping the epithelial surface as before and mounting the product in iodized serum. The preparation so obtained is similar to that already seen of the fresh epithelium, but the separated cells are much more numerous, and at the same time more perfect, for the intercellular substance which unites them being softened and dissolved by maceration in the serum the cells are more readily obtained free.

The snip which was placed in osmic acid must be left for two days; it is then placed in water for an hour or two to remove the excess of the acid. To examine the tissue remove a minute portion and break it up as finely as possible with needles in a drop of glycerine on a slide. The cells will be found to exhibit the same general characters as regards form and appearance, but they are stained of a dark grey colour, and in consequence appear for the most part much more distinct. Any fatty particles which they may contain are coloured intensely black. This preparation possesses the advantage over the others that it can be preserved. All that is further necessary is to apply a little fixing cement of some sort around the edges of the cover-glass.

The kind of fixing cement used for preparations mounted in glycerine is immaterial; a solution of hard Canada balsam in chloroform, dammar varnish, gold-size, or Brunswick black may be employed with almost equal advantage; but it is important to see more particularly to two points before applying it. The first is that there shall be just enough glycerine in the preparation to fill up the space between the cover-glass and slide, neither too much nor too little; any excess must be removed with blotting-paper. At the same time one or two small air-bubbles in the glycerine do no harm, unless they are so situated as to obscure the tissue; on the contrary, by yielding and becoming somewhat compressed when the glycerine tends to expand from being exposed (in summer, for instance) to a warmer temperature, they may tend to prevent that fluid from bursting its bonds and exuding through the enclosing cement. The second point to be attended to is that the slide where the cement is to be applied is not wetted with glycerine, for this would prevent the adherence of the cement.

A word may here be said as to the course to be adopted should any glycerine have accidentally got on to the upper surface of the cover-glass. No attempt should be made to wipe it off at once, for the cover-glass will be pressed upon and moved, and the preparation will probably be crushed and spoiled; but time should be allowed to the fixing cement to become thoroughly hard, and then the glycerine can be removed without danger to the tissue by means of a camel-hair pencil moistened with distilled water.

For the present it will be sufficient to conclude the

study of epithelium with the description of the best modes of viewing ciliated epithelium, and of studying the action of various reagents upon the ciliary motion. The other more specialized forms of epithelium, which are found in glandular organs and elsewhere, will be seen and studied when the several organs and parts in which they occur are prepared.

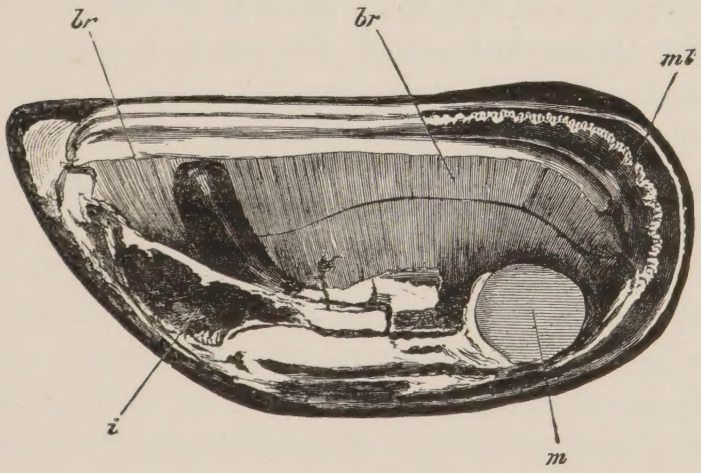
Preparation 7. Ciliated Epithelium in its living state may be readily obtained from the mouth and gullet of the recently killed frog. A drop of the aqueous humour should first be collected by passing a capillary glass tube through the cornea into the anterior chamber of the eye; the drop is placed upon a slide, and then, the frog's mouth being held open by an assistant, the roof is gently scraped with the point of a clean scalpel, so as to remove the adherent mucus. A little of the epithelium will be brought away with this, and on placing it in the fluid, and covering the preparation (taking the precaution of previously placing a hair in the drop), the cells may be sought for with a high power. For the most part they will be collected into little groups of three or more, the cilia being in active movement and producing currents in the liquid, so that free particles, blood-corpuscles, and other small objects are moved along in it. But if the group is small, or especially if entirely isolated cells are seen, it will generally be found that the cilia act upon the pieces to which they are attached like little paddles, moving them about in the fluid. The cells, it may be observed, are either shortly columnar or are spheroidal; the nucleus is seldom distinct, because concealed by the granular nature of the cell-protoplasm. The cilia themselves can best be seen when they are moving languidly or when their motion has altogether stopped; they are very fine, and spring, a number together, from the base or free surface of the cell.

Preparation 8. Cilia of Mussel.—But by far the most convenient object for the study of ciliary motion is to be found in the gill of the common seawater mussel (*Mytilus edulis*).

Here the cilia are very large, and their motion will go on unimpaired for many hours. Hence they are particularly well suited for the observation of the action of most of the reagents which affect ciliary movement.

One or more mussels may readily be procured at any fishmonger's ; those only should be chosen which remain tightly closed, for those with open valves are in most cases already dead. One of the shells may then be forcibly separated by means of a knife, when the gills (fig. 16, *br*) will come into view, as flattened expansions of a yellowish colour, covering a considerable part of the shell, inside

FIG. 16.



Valve of mussel, showing *br*, *br*, the expanded gills or branchiæ, which, owing to the little bars of which they are composed, present a striated aspect.

ml, mantle ; *m*, cut adductor muscle ; *i*, mass of viscera ; the dark projection just above is the foot.

its lining membrane *ml*. By observing carefully it may be noticed that they have a striated aspect, the markings passing transversely to their length, and by taking up a small portion with a forceps it will further be seen that this striation is due to the fact that the gill is made up of a number of little bars which are distinct from one another for the greater part of their length. Take now a small piece of the gill, including three or four of the bars, and placing it upon a slide in a drop of the seawater which the shell always contains,

separate the bars one from another by means of needles ; the preparation may then be covered and observed.

Each of the bars in question will be seen to be fringed with large cilia, which are set at an appreciable distance apart along nearly its whole length, but at the free extremity of the bar are much more densely arranged. Those in this situation resemble in appearance the cilia of the frog's mouth, with the exception that they are very much longer ; and like them they appear to spring a large number from each cell, whereas the others are stiff-looking and obviously thicker, and are connected at their base each to a single, comparatively small epithelium cell. In spite of these differences of appearance and attachment the two kinds seem to be essentially alike in nature, for the mode in which they move is similar and they are similarly affected by reagents.

It will be found that after a preparation such as that just described has been made for several hours the movement will have become somewhat languid, and then the manner in which the individual cilia move can be more clearly made out. The preparation can be used also for the study of those agents which tend to revive and stimulate ciliary motion, and it will be seen that it is precisely the ones which most accelerate the amœboid movements of the white blood-corpuscles that have the most marked effect upon the cilia also.

Preparation 9. Action of Warmth upon Ciliary Motion.— The same mode of applying heat to a preparation of cilia is to be used as was employed for observing the effect of warmth upon the blood (p. 18). It is well, after enclosing the preparation with oil (or, what is better, with melted paraffin) in the manner there detailed, to put it aside for some time, when it will probably be found, as just stated, that the movement of the cilia is languid or altogether arrested. On now gradually warming the preparation the motion becomes revived, and as the heat is raised becomes, *pari passu*, faster and faster, until a point is reached at which the tissue is injured by the high temperature, when the movement stops

altogether and is not again resumed. But if the experiment be stopped short of this point and the source of heat removed, it will be seen that, conversely, as the temperature of the stage falls the rate of movement also diminishes, until, when again quite cold, the cilia may again almost stop, although they can be made to resume their active motion on again applying warmth.

Preparation 10. Action of Weak Alkalies.—A very weak solution of caustic potash in salt solution, like that which was used in investigating the effect of weak alkalies upon the blood, is to be applied to a preparation of cilia which have become somewhat languid, in exactly the same manner as in the case of the blood—by allowing a little of the fluid to pass in at the edge of the cover-glass and diffuse itself with the seawater, so as to come gradually in contact with the slowly-moving cilia. The action is immediate; the cilia revive and vigorously lash the liquid into which they project, but the effort is soon exhausted, for the alkaline liquid penetrating the cells destroys their vitality, and the motion of their cilia stops beyond recovery. The stimulant action is not, however, peculiar to weak alkalies, for it is exhibited also by acids and by many other substances which, applied concentrated, would instantly destroy the tissue, but when much diluted tend to revive and for a time maintain accelerated the ciliary motion.

Preparation 11. Action of Carbonic Acid Gas.—This reagent is to be applied to a cilia preparation in the gas-chamber in the manner directed for the investigation of its action upon the newt's blood-corpuscles (p. 35). Everything being ready, choose a part of the preparation where the cilia are not acting very vigorously, and whilst still watching allow the gas to pass over the preparation. Its immediate action is seen to be that of a weak acid—that is to say, the rate of movement, if not already at its fastest, becomes accelerated—but as soon as the oxygen of the air in the chamber is entirely displaced by the continued stream of carbonic acid the motion ceases

altogether. As soon as this result is obtained cut off the stream of CO_2 and reverse the experiment by blowing air in at the side-tube, and thus displacing the carbonic acid from the chamber. The motion will almost instantly recommence. This shows that it was the absence of oxygen and not the presence of CO_2 which produced the stoppage of movement; for there is, of course, an appreciable quantity of carbonic acid in the air which is thus blown from the mouth into the chamber.

Preparation 12. Chloroform.—The gas-chamber is again used for this reagent, the apparatus being arranged in the way previously recommended (p. 27). Choosing a part of the preparation where the ciliary motion is vigorous, gently blow a stream of the mixture of air and chloroform vapour from the bottle into the moist chamber. The cilia become gradually slower and eventually stop. Now slip the india-rubber tube off the bottle, and gently blow air through the chamber, to displace the chloroform vapour. The cilia will slowly revive on the readmission of air, and will soon be found to work as vigorously as ever. Like that with the carbonic acid this experiment can be repeated a number of times with a like result, if the chloroform vapour is not allowed to remain too long in contact with the preparation.

Preparation 13.—To study the characters of the individual cells a portion of membrane which is covered by ciliated epithelium is macerated in some fluid which softens and dissolves the intercellular substance, whilst preserving the cells themselves. The best for this purpose is a weak solution of bichromate of potash (1 in 800). A large quantity of this must be employed, and the tissue—a piece of the trachea of a rabbit or other animal, for instance—is left in it for about forty-eight hours. With the point of a scalpel a little of the epithelium is then gently scraped from the inner surface, and being placed in a drop of distilled water on a slide, is broken up with needles as finely as possible. A small piece of hair is placed in the drop, to prevent the soft, delicate cells

from being crushed by the weight of the cover-glass. This is now superadded and the preparation carefully examined with a high power.

Numerous completely isolated cells are seen floating in the liquid, and these preserve for the most part their natural form and retain their cilia, although the latter are, of course, no longer in motion. The bright border through which the cilia appear to pass, the faintly granular cell-substance, the clear nucleus with bright nucleolus in the middle, and the tapering, branched extremity of the cell are, in most, clearly visible. Besides these single cells others are present which are still united in groups or patches, in which, when viewed from the surface, the bases of the cells have a mosaic appearance. Moreover, a few 'goblet' cells may here and there be met with, no doubt produced in a similar manner to those found amongst the columnar cells of the intestine and elsewhere, viz. by a swelling up of the mucus contained within the cells when these come in contact with a watery fluid. In these cases the base of the cell is ruptured and the cilia are destroyed.

Besides the ciliated cells certain others may be seen in the specimen which are of an irregular and usually elongated shape, with pointed and often branched extremities, and are destitute of cilia. They lie, in the natural state, between the ciliated cells, but their position can only be properly studied in sections of the mucous membrane.

If it be wished to permanently preserve such a preparation as that now under description, it is necessary first to stain the cells somewhat and then to substitute glycerine for the staining fluid. Either dilute logwood or one per cent. osmic acid solution may be used for staining the cells. The former colours their nuclei very intensely, the latter gives a uniform grey tint to the cells. The colouring fluid is applied in the following manner:—A little is taken up into a pipette and a drop is then brought in contact with one edge of the cover-glass. The pipette is then removed and at the other edge a

small piece of filter-paper, torn into a triangular shape, is placed, with the apex of the triangle touching the cover-glass, so as to draw the fluid slowly through. When the staining fluid has replaced the water in which the preparation was made the filter-paper is removed and the preparation is left until the cells appear sufficiently coloured, a little more fluid being occasionally added if there seems any danger of the specimen becoming dry. With a solution of logwood, even though very dilute, a few minutes suffices; the osmic acid solution should be allowed to remain an hour in contact with the cells. The staining fluid is replaced by water in the same way, and finally, the filter-paper being removed, a small drop of glycerine is applied at one border of the cover-glass and gradually takes the place of the water as this evaporates at the edges. In a day or two the cover-glass may be fixed with chloroform balsam.

CHAPTER III.

CONNECTIVE TISSUE.

Areolar Tissue.—In the areolar tissue and in connective tissue generally there are several parts which present themselves for study; and in order to observe each to the greatest advantage different modes of preparation are for the most part requisite.

Preparations 1, 2. The Fibres of Areolar Tissue.—For the observation of the fibrous elements simply, without special regard to their arrangement or relation to the other elements, all that is necessary is to place a small portion of areolar tissue, taken from any part, on the centre of a glass slide, just moistened with salt solution, and with clean, sharp needles separate it as finely as possible into filamentous shreds. Then, before there is time for the preparation to become dry, place a drop of salt solution on a cover-glass and invert this over the tissue. The object of using but very little fluid to prepare the tissue in is to prevent the filaments from running together and becoming entangled when released from the needles.

In a preparation so made nothing is as a rule apparent save the wavy bundles of connective tissue fibrils, these when much developed obscuring, by their effect upon the light, the elastic fibres and corpuscles of the tissue. But if now a second preparation be made in precisely the same way, except that, in place of salt solution simply, salt solution containing one part of acetic acid in 200 is placed upon the cover-glass, and if then the object is immediately examined with a high power, it is seen that the fibrils which compose the bundles

have become indistinct, whilst the bundles are much swollen, except, it may be, at intervals here and there. At the same time certain other fibres, almost equally fine but more sharply defined than the white fibrils, and always running singly, never in bundles, come into view. These are the elastic fibres. If one of them be followed for a short distance it will probably be seen that it sooner or later gives off a branch which unites it with a neighbouring fibre, whereas the white fibrils never show any disposition to branch or unite with one another, but those in each bundle maintain from end to end a perfectly parallel course. The elastic nature of the filaments which are brought into view by acetic acid is shown, in such a preparation as we are describing, by the fact that wherever in the process of teasing the tissue they have become broken across, the fibres have, by the recoil from the stretching to which they were submitted before the rupture occurred, been thrown into bold curves, especially marked near the broken extremities, which are often recurved. That this curved or coiled appearance of the elastic fibres, although highly characteristic, and always observable when the tissue is thus prepared, is, however, not a natural one, is shown by the fact that, as will immediately be described, when precautions are taken to preserve as much as possible the normal arrangement of the tissue-elements the elastic fibres are seen to pursue a rectilinear course.

Preparation 3. The Corpuscles of the Areolar Tissue.—To demonstrate the cells or connective tissue corpuscles the preparation is made more methodically. A film as thin as possible must be obtained for observation, so as to avoid the necessity of tearing the tissue. Such films are naturally present in the areolar tissue of most parts, and may be seen when the organs which it connects are gently drawn asunder from one another, as, for instance, when the skin is raised and reflected from the subjacent fasciæ and muscles. The most convenient source of such a delicate film is to be found in the exquisitely thin and transparent tissue which invests

and lies between the muscles of the fore-limb of the rabbit and guinea-pig. The tissue in this situation, especially if taken from a young animal, is devoid of fat and not so completely overridden by the bundles of white fibrils but that the elastic fibres and the connective tissue corpuscles can be made out even without the addition of reagents. The mode of preparation is as follows:—

The animal having been killed by bleeding, the skin is snipped through around the upper part of the fore-limb and is then forcibly reflected from the limb. In this operation care must be taken to avoid besprinkling the subjacent parts with the cut hairs of the animal. A piece of the tissue over or between the muscles is then seized with the forceps and snipped off with sharp, fine scissors. The snipped-off tissue shrinks immediately around the end of the forceps and appears very unsuited for microscopical examination. But place it on a clean slide, without the addition of any fluid, and with a pair of mounted needles endeavour, by drawing out first this corner and then that, to again reduce the gelatinous-looking piece to the condition of a thin film, and it will be found that this can be effected without much difficulty, for when not floated up by fluid the thin edges of the film tend to stick to the glass, and cease to shrink away from the position to which they are drawn by the needles. At the same time, whilst it is important not to *add* fluid to that which naturally moistens the piece of tissue, it is equally important never, during the whole process of stretching, to let the film become actually desiccated, for this would altogether ruin the tissue for microscopic purposes. The best way to prevent such an untoward result from happening is to breathe now and then on the object whilst it is being prepared; by so doing needless haste will be avoided and more time and pains can be taken for the complete display of the film. This being effected, a cover-glass (which should have been previously cleansed and placed in readiness) is taken, wetted on its under surface with a drop of salt solution, and quickly superposed over the film

of tissue, which is thus prevented from shrinking up again into a shapeless mass. The fibres, both white, (in wavy bundles of various sizes) and the elastic, and corpuscles may now be carefully observed, at first with the usual high power and afterwards with the highest obtainable, and some of the corpuscles should be sketched. Moreover, search may be made for lymph or pale blood-corpuscles, a very few of which are generally to be found in the connective tissue ; they are readily distinguished from the fixed corpuscles of the tissue by their small size —small, obscure, and generally multiple nuclei—and especially their amœboid movements, of which it is probable no trace will be apparent even to the most assiduous observation in the connective tissue cells proper.

Preparation 4.—Although both corpuscles and elastic fibres may be seen in a preparation of this kind made with an indifferent fluid, they are better seen if the white fibres are acted upon by acetic acid, and still better if this action is combined with that of some staining reagent, so that the corpuscles are brought more prominently into view. Moreover, the preparation admits of being permanently preserved in glycerine after such a method of treatment. Up to a certain stage the procedure is the same as that above described, but instead of placing salt solution upon the cover-glass, before inverting it over the film, a solution of acetic acid (1 per cent.), coloured by the addition to it of one-third its volume of logwood-alum solution, is employed. The object is examined as quickly as possible after the application.

The connective tissue corpuscles can probably be made out already in the thinner parts of the preparation, with their clear oval nuclei and the flattened irregular area occupied by their cell substance. In a few minutes the nuclei will be tinted by the logwood, and will then show up much more prominently ; but to get the cell-bodies sufficiently coloured it will be necessary to leave the staining solution half an hour or more in contact with the preparation. Meanwhile, to obviate the effects of evaporation, a considerable drop of the

colouring fluid should be placed on either side in contact with the edges of the cover-glass. The excess of fluid, moreover, has a tendency to raise the latter slightly from the film of tissue, and in this way a more ready access of fresh colouring fluid is permitted. When it is found on examination that the corpuscles are properly stained the solution may be drawn off by a slip of filter-paper applied to the edge of the cover-glass on one side, whilst to the other a drop of distilled water is applied, the logwood being for the most part in this manner rinsed away. The filter-paper is then removed, and a drop of glycerine placed in contact with the edge; this, as the water evaporates, will slowly diffuse itself under the cover-glass and take its place. It is well to put the preparation aside until the following day, when the process will be completed; all that is then necessary is to draw a camel-hair pencil wetted with chloroform balsam around the edge of the covering-glass, so as to fix it securely in its place.

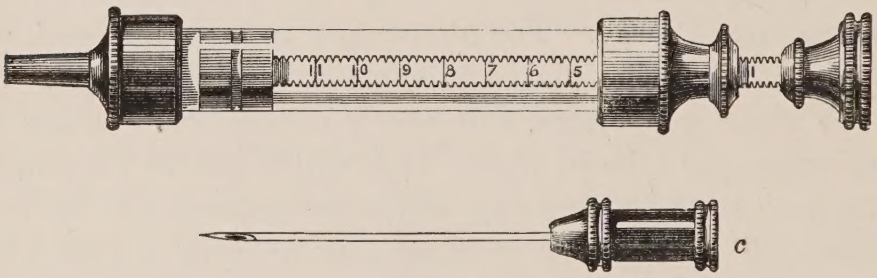
In specimens treated with acid there may be observed a constricting ring at intervals along the course of some of the connective tissue bundles, an appearance which has long been familiar to histologists, but the cause of which is not yet clearly determined, some supposing it, from its resistance to the action of acids, to be of the nature of elastic tissue; others that the appearance is caused by the process of a corpuscle enwrapping the bundle; the latter opinion being mainly founded on the fact that the ring becomes stained by certain reagents which also colour the cells. For example, as shown by Ranvier, the constricting ring is tinted red by picrocarminate of ammonia, whilst the elastic fibres are coloured yellow.

Preparation 5.—In most of the larger animals (*e.g.* the dog), the connective tissue is, in the adult, so densely pervaded by the bundles of white fibres as to render it impossible to obtain a film delicate enough for easy observation without tearing the texture with needles, and thereby distorting the cellular elements. Or, on the other hand, it may be desired even in those animals in which such delicate tissue as that the preparation of which has just been

described is found to obtain a specimen from a part where the connective tissue is not naturally extended in so advantageous a manner for preparation and observation. In these cases the following method may be employed with advantage :—

In a recently killed dog a flap of skin is dissected back, and a Pravaz or other hypodermic syringe (fig. 17), provided with a

FIG. 17.



Pravaz syringe for interstitial injections; *c*, the cannula. The syringe is glass with ebonite fittings; the cannula is a fine perforated steel needle set in ebonite. Natural size.

fine cannula, having been previously filled with salt solution, the point of the cannula is inserted underneath the layer of connective tissue which is most superficial on the reflected portion of skin, and a little of the fluid is forced out. This does not immediately diffuse itself uniformly through the loose areolar tissue, but remains for a short while at the same place, forming a little bulla of liquid bounded and covered in by a film of tissue, the thickness of which depends upon the depth to which the cannula was inserted. If it does not appear thin enough a second attempt should be made at another spot. Then, before the bulla has time to subside—that is to say, before the fluid has time to diffuse itself through the meshes of the tissue—snip off the whole projection with a single cut of a pair of scissors, which for this purpose should be particularly sharp and clean, and transfer the snipped off portion to a clean slide. Here it may either be at once covered in salt solution and examined without reagents, or

may be treated with acetic acid, logwood, &c., as in the mode of preparation just described.

Preparation 6.—A modification of the above method consists in injecting into the tissue a solution of gelatine instead of salt solution. The gelatine solution is made by taking some very clear French gelatine, allowing it to soak for an hour in water, and then, after pouring off all the excess of that fluid, placing the soaked gelatine in a beaker over a water-bath until it is entirely melted in the water which it had imbibed. The syringe is then warmed by immersing it for a minute or two in warm water, and is filled with the gelatine solution, and a little of this is injected into the subcutaneous connective tissue, so as to produce a bulla like that made by the salt solution. In cold weather the gelatine will set almost immediately ; in warm weather the process may be accelerated by surrounding the bulla with small lumps of ice.

When the gelatine is quite firm sections of it are to be made with a razor. As they are cut they may be placed in salt solution.

Before mounting them it is well to stain the specimens, and one of the best staining fluids for the purpose is a watery solution of magenta. This colours the elastic fibres strongly, the corpuscles distinctly, and the bundles of white fibrils slightly, while the gelatine which was injected and of course occupies all the interstices is hardly stained at all. The time of immersing the sections varies of course with the strength of the fluid, but this should not be too highly coloured, and it can be then seen without much difficulty when the sections are sufficiently stained. They are subsequently placed in water for a minute or two to remove the excess of magenta prior to transferring them to a slide. A drop of glycerine is now added and the cover-glass laid on, after which the slide is gently warmed over a small flame or otherwise until the gelatine in the sections just melts so as to allow the cover-glass, which was probably tilted up somewhat owing to the thickness of the sections, to settle down. The specimen

may then be examined, and if satisfactory, may be preserved, the preparation being completed after a day or two by fixing the edges of the cover-glass with chloroform balsam.

By the modes of preparing connective tissue already described the bundles of white fibres, the elastic fibres, and the corpuscles are brought under observation, and it would seem at first sight that these of themselves entirely constitute the tissue. But in considering the nature of the films obtained—that they are, namely, continuous over a greater or less area—it is clear that the presence of fibres and cells is not alone sufficient to account for the laminæ which are spread upon the slide. And, indeed, by closely observing the preparation it will be apparent that there is pellucid substance uniting everything together, through which the fibres run, and in which the corpuscles lie embedded. There is, it is true, a difficulty in making this out in most parts, in consequence of its extreme clearness, and the fact that its refractive index is little different from that of the watery fluid the tissue is examined in; moreover, in the logwood preparations the intermediate substance remains entirely unstained. Nevertheless, towards the edge of the preparation, where a comparison can the better be made with the surrounding fluid, the fact that such a clear intermediate substance does really exist will be sufficiently evident; and the more so if the cover-glass be slightly moved, or one edge be gently pressed down with a needle. But we possess in the silver method of Recklinghausen a ready means of demonstrating its existence in an obvious manner; for the ground substance (or intercellular substance) of the connective tissue, and, indeed, of almost every other tissue in the body, possesses the distinctive property of reducing the salts of silver under the action of light, so that the metal is deposited in it either in a free state or, more probably, in combination. The effect of this deposition is, that the ground substance becomes stained of a colour varying with the intensity of the light employed, and with other conditions, from a light brown to a brownish black. The fibrous elements participate for the most part in this staining, and are frequently, especially when the preparation has been, as is usual with silver preparations, mounted in glycerine, indistinguishable from the ground substance through which they course, and which also unites the white fibres into the bundles which they form. The cellular elements, on the contrary, remain absolutely unstained, and, moreover, after the action of the silver salt are no longer affected by those

staining fluids which otherwise have a particular affinity for them; it is therefore no longer possible to bring them into view. Wherever, then, a cell is situated, there appears after the reduction of the silver nothing but a white patch upon or in the brown ground; and if, as is not unfrequently the case, several flattened cells may have occurred together with their edges in juxtaposition, the group appears as a larger white patch intersected by dark lines, these representing a small amount of intercellular substance between the individual cells. The appearance is similar to what is observed in an epithelial tissue after the silver treatment, for in this the intercellular substance is always very small in amount. Such an arrangement of connective tissue cells is on this account designated 'epithelioid.' The white patches, then, in the silvered preparation of connective tissue represent either depressions on the surface of, or actual cavities within, the matrix or ground substance, containing cells, which themselves are not visible, so that the white patches are termed the *cell-spaces*, or (recalling the analogous case of bone) the *lacunæ* of the connective tissue.

It is the more appropriate to give them a special designation, because, as may be made out by a careful comparison of specimens of connective tissue from the same part, some prepared with logwood or chloride of gold, to show the cells, others with silver, to show the cell-spaces, the cell-spaces are in many cases distinctly larger than the cells, and may also be of a somewhat different figure; they are not necessarily, therefore, as has sometimes been supposed, and as is no doubt the case with the clear parts of a silvered epithelium, merely the cells left white. The difference in the relative size of the cell-spaces and the contained cells obtains no doubt more frequently, or at least can be more readily made evident, in the firmer varieties of connective tissue, where the ground substance is everywhere pervaded with fibrous bundles, and has in consequence lost its soft and yielding nature, which otherwise permits it to adapt itself more readily to the shape of the cells. Moreover it must be remembered that the natural course of the lymph in the tissue is around the cells, between them and the enclosing ground substance; and when from any cause that fluid is in excess the effect will be that the cell-spaces are more distended and appear preternaturally large.

This much having been said in order to explain the appearances produced by the silver method of treatment—appearances which have been called in question, and their

value as yielding evidence of any constant structure in the tissues altogether denied by histologists of considerable eminence, but, as it must now be admitted, without sufficient grounds—the best mode of applying it to ordinary connective tissue, such, for instance, as the subcutaneous, may now be described.

Preparation 7.—The skin of a recently killed rabbit or guinea-pig [1] having been stripped off one of the limbs, this is disarticulated at the proximal joint, and is rinsed for a second or two in a beaker of distilled water, in order to wash away any blood or lymph, which might happen to be on the surface, and which would cause a granular precipitate with the nitrate of silver solution. The latter, a solution of 1 part of the salt to 200 of distilled water, is then either poured over the surface or dropped on it from a pipette. After two or three minutes the silver solution is quickly washed off by a stream of distilled water, and the limb is then at once placed in a beaker of spirit, and exposed to direct sunlight, or, failing this, to bright diffused daylight. In a few minutes in the sunlight, and after a longer time in diffused daylight, the silvered surface will have acquired a uniform brownish tinge to the naked eye. When the colour is strongly marked it is as well to remove the beaker from the light, lest the preparation become too darkly stained. The limb should be allowed to remain in the spirit twenty-four hours; at the expiration of this time it is placed in a dish, and, by the aid of fine forceps and scissors, a piece of the superficial stained layer is dissected off under spirit. In doing this care must be taken not to drag at all upon the membrane which is

[1] These animals are selected because there is likely to be less fat in the subcutaneous tissue than in that of the cat or dog or other animals commonly used in the laboratory. It is important to remember that any tissue which is to be submitted to the silver method must be fresh and unacted upon previously by any reagent whatever, since this would entirely prevent the desired effect; moreover, if blood have accidentally got on to the part it must first be rinsed away with distilled water.

removed, so as to throw it into creases. The piece is then transferred to water, and floated on to a clean glass slide with the browned surface of the membrane uppermost. The slide is then carefully removed from the water, the portion of tissue being kept flat on its upper surface; and most of the superfluous water having been wiped away, a cover-glass, on which a drop of glycerine has been placed, is inverted over the preparation. Before putting on the cover-glass it is well to examine the object under a low power, in order to make sure of the absence of folds and creases or specks of dust upon it: if any such be seen they must be carefully removed with a needle. Indeed, it may be recommended as a golden rule in making histological preparations never to put the cover-glass in its place until a glance at the object under a low power of the microscope has certified the absence of any marked imperfection: if this be attended to the time will often be saved which would otherwise be spent in mounting worthless specimens.

These silver-preparations of the subcutaneous tissue vary considerably in appearance according to the part of the limb from which they are taken, and it is in many cases difficult fully to comprehend the meaning of what is seen, so that it will be better perhaps to omit for the present this preparation, especially as the one following furnishes a very ready and clear means of demonstrating the cell-spaces of connective tissue.

Preparation 8.—The connective tissue which covers the tendons of the superficial extensor digitorum of the ox's foot as they run through sheaths formed by the tendons of the deep extensor is much more easily prepared by the silver method than the looser kinds like the subcutaneous just described. A piece of such a tendon is taken, rinsed in distilled water to remove the synovial fluid which covers it, and treated with silver solution in the way just described; and is then, after washing, placed in the light in strong spirit. It soon becomes brown, when it may be removed from the light; and after remaining twenty-four hours in the spirit it is

easy, with a sharp knife or razor wetted with spirit, to obtain a thin surface section. This, after being immersed for a minute or two in water to get rid of the spirit, is mounted in glycerine with the browned surface uppermost. It should present, if successful, an extremely characteristic and beautiful image of white branched cell-spaces, single or in groups, upon a brown ground.

In both the preparations last described it is possible to show the nuclei of the corpuscles which lie in the cell-spaces by subsequent staining with logwood. Sometimes they are visible even without this treatment.

Preparation 9. Elastic Network.—The proportion of elastic fibres varies considerably according to the part from which the connective tissue under examination is taken. Some serous membranes contain a large number of elastic fibres; and since they are readily spread out in their natural condition the network which these fibres form by their branchings and conjunctions is easily made evident. One of the best objects for this purpose is to be found in the rabbit's mesocolon. A piece of this, moistened with a little salt solution, may be spread out as flat as possible upon a slide, and a drop of dilute acetic acid (1 per cent.) having been placed on a cover-glass, this is inverted over the tissue. The white fibrils quickly swell up and become indistinct, whilst the elastic network comes clearly into view. The preparation may be made permanent by putting, as in former preparations, a drop of glycerine at the edge of the cover-glass, and after this has had time to diffuse itself, cementing the edges with chloroform balsam as usual.

Preparation 10. Elastic Tissue.—The elastic ligaments are to be regarded merely as connective tissue structures in which the elastic elements of the tissue preponderate. There is always a quantity of ordinary areolar tissue amongst the elastic fibres even here, but not sufficient to obscure them, especially as they are generally of larger size as well as in greater number than elsewhere. It is sufficient, in order to

see them, simply to teaze out a portion of the ligamenta subflava of the vertebræ or other elastic tissue in water or salt solution. If it be desired to keep the preparation it can be mounted in glycerine.

Preparation 11. Section of Elastic Fibres.—To observe the shape of the fibres a transverse section may be made of a piece of ligamentum nuchæ of the ox, in which the fibres are extremely large.

In order to obtain the requisite firmness for cutting, place a small piece of the ligament in a quantity of two per cent. solution of bichromate of potash for fourteen days; then place in water for two or three hours, and transfer to spirit; in a day or two sections across the direction of the fibres may be made with a razor, the surface of which should be wetted with spirit. The sections are to be placed in water for a minute or so, and finally mounted in glycerine.

Preparation 12. Fibrous or Tendinous Tissue.—This may be examined by separating a small shred from a tendon or ligament, and teazing it out as finely as possible into its constituent bundles. The operation is conducted with the aid of needles, the tissue being placed in a drop of salt solution, and it is first examined in this, being afterwards treated with dilute acetic acid and logwood (see Prep. 4). But it is a troublesome matter to make the separation fine enough without disturbing too much the arrangement of the cellular elements of the tissue. Fortunately we can obtain, from the tail of the mouse or rat, tendons which are, so to speak, naturally dissociated; for excessively fine tendons run along the whole length of the tail, and can readily be drawn out, needing no further manipulation than is necessary to place them advantageously under the microscope. The following is the mode of procedure:—

Preparation 13.—In a recently killed mouse the tail is seized about half an inch from the tip between the thumb-nail and fore-finger of the right hand; and the delicate skin being partially nipped through and the vertebral column

broken at this point by the pressure of the nail, it will be found quite easy, the base of the tail being fixed by the left fore-finger and thumb, to separate the end altogether and drag it away from the remainder of the tail. In doing so it will be found that the minute tendons which are attached near the tip, owing to their comparative toughness and strength, are not broken through at the spot in question, but are dragged out of the channels in which they run, and may in this way be obtained in the form of a bundle of exquisitely fine silky threads, which are to be immediately immersed in a glass dish of salt solution. Now cut away two or three of the fine threads with sharp clean scissors, and seizing them by one end with fine forceps, or leading them with a needle-point, float them on to a glass slide which is held immersed in the fluid, and is then carefully lifted out. After arranging the minute tendons as nearly straight as possible on the slide and blotting up most of the superfluous salt solution or allowing it to run off, place a short piece of hair beside them, to avert the pressure of the cover-glass, which is now placed over the middle of the threads in such a way that, since they are considerably longer than the width of the cover-glass, their ends project beyond on either side. The object of this is to permit them to be drawn straight with needles should the superposition of the cover-glass have displaced them. These ends, moreover, since they are exposed to the air, soon dry and stick to the slide, so that subsequent treatment with reagents does not tend to displace the tendons, which are thus maintained in an extended condition. Examined thus in salt solution, little is visible beyond the slightly wavy, closely packed white fibrils, collected, as longitudinal streaks seen here and there indicate, into a few indistinct bundles. But allow a little dilute acetic acid (1 part of the glacial acid to 200 of salt solution) slowly to pass under the cover-glass, and a remarkable change becomes apparent. As the acid reaches the tendons, they slowly swell up and become more transparent, the fibrils becoming indistinct; and

now chains of small oblong faintly granular cells, each with a clear nucleus situated near one end of the cell, and often opposite that of a neighbouring cell, come into view. These are the tendon-cells, the corpuscles of the fibrous connective tissue; only the central thicker portion of each, which lies in the interstice between three or more tendon bundles, is seen at present; the thin lamellar prolongations, which extend between two tendon bundles, are too delicate to be made out without staining. In some of the chains a bright longitudinal line is to be seen on each cell; this appearance is merely produced by a lamellar prolongation of this sort, which happens to extend vertically to the plane under observation.

After the action of the acid has been prolonged for some time, the cells gradually lose their distinctness, and eventually can with difficulty be made out, although the fibres are more swollen and indistinct than ever. But if a little of the coloured acetic acid which was used for the subcutaneous tissue (Prep. 4) is allowed to run under the cover-glass, first the nuclei, and then the bodies of the cells and their prolongations become coloured, whilst the fibres remain unstained, just as in the parallel case of the areolar tissue.

When the colouration is sufficiently deep, the staining fluid may be replaced by water, and this in the usual way by glycerine; and finally, the edges of the cover-glass being cemented, the preparation can be permanently preserved. Examined with a high power, the tendon-cells now appear in the successive horizontal planes as quadrangular flattened bodies, thickest near the middle, and gradually shading off at either side, and marked with one or more dark lines running longitudinally, which are, in fact, the bright lines to which attention was previously drawn, and which have now become stained. To show that these are actual flattened extensions of the cell, and not mere markings, it is necessary to compare the appearances presented by a transverse section.

Another method of displaying the cells of tendon is that originally employed by Ranvier. One or two of the small tail tendons are

placed on a slide, and their ends are fixed with paraffin, so as to keep them extended. A few drops of a one per cent. solution of picrocarminate of ammonia are then placed upon them and left for half an hour, after which the picrocarmine is washed away with distilled water and the tendons are mounted in glycerine, acidulated with acetic acid.

The picrocarmine is made by adding to a saturated solution of picric acid a strong solution of carmine in ammonia, to saturation, evaporating the mixture to one fifth part of its bulk, allowing to cool, filtering from the deposit which occurs, and evaporating the filtrate to dryness over a water-bath; when the picrocarmine is left as a crystalline powder of an ochre red colour.

Preparation 14. Transverse section of Tendon.—To obtain this, it is best to take a large tendon, for it is much easier to get transverse sections of such a one, and in all essential points of structure it is quite similar to the minute tendons which, for the sake of convenience, we have just been employing. A piece, then, of any tendon, large enough to be grasped by the fingers, is placed in strong spirit for a day or two. This gives it a very hard, horny consistence, and it is easy, with a sharp knife or razor wetted with the spirit, to get one or two thin sections from the end. These are placed on a slide, a drop of the acidulated logwood is added and left in contact with them until they are sufficiently stained. It is then carefully washed away by a drop or two of water applied from a pipette; and finally a little glycerine is placed upon a cover-glass, which is then inverted over the preparation.

It will be seen that the tendon is divided into fasciculi by septa of areolar tissue, the corpuscles of which (seen edgeways) are brought into view, being stained by the logwood; it will further be observed, if the sectional area of one of the smaller fasciculi is attentively examined with a high power, that it again is divided (although incompletely) into several still smaller bundles by the branching processes of deeply coloured stellate bodies situate at the angle of junction between three or more such bundles, and extending a greater or less distance between the neighbouring bundles.

These stellate bodies, with their processes, are the tendon-cells with their lamellar extensions, as seen in section; the smaller fasciculi, which are separated by areolar tissue, corresponding with the whole of one of the caudal tendons of the mouse or rat.

Preparation 15. Cell-spaces of Tendon.—To show the cell-spaces in which the above-mentioned tendon-cells are contained:—

As before, break off the end and draw out some of the tendons of a mouse's tail (that previously used will still yield a sufficient number). Then hold the tendons in a shallow dish of distilled water, and with a medium-sized camel-hair pencil brush them firmly from end to end six or eight times. Remove them now from the water, and immerse them in a large watch-glass of nitrate of silver solution ($\frac{1}{2}$ per cent.) for fifteen minutes; then place in a glass vessel of water and expose to sunlight. As soon as they are well browned, pieces may be cut off, laid straight in water upon a slide, covered, and the water then drawn off and replaced by glycerine; after which the cover-glass may be fixed in the usual way.

Preparation 16. Epithelioid covering of Tendons.—The object of first brushing the tendons is to remove the layer of flattened cells which covers the surface of each, and which, if allowed to remain, prevents the silver solution from properly acting upon the deeper parts of the tissue. To show this layer, another set of tendons may be treated with the silver solution in a similar way, but with a minute's immersion and without previously brushing them, when it will probably be found that the superficial epithelioid stratum is alone apparent.

Preparation 17. Adipose Tissue.—The simplest way of showing the fat vesicles is by teasing out a small portion of the tissue in a drop of salt solution; taking the precaution of putting a narrow slip of blotting-paper on either side to avert the pressure of the cover-glass.

But a far better way of demonstrating the structure of the

tissue generally, consists in the employment of the method of interstitial injection with gelatine (see Preparation 6, p. 61). The gelatine is injected into the interior of one of the fat-lumps, and sections are made and treated in the way as described. By this method the fat-cells are somewhat separated from one another, and all their parts, as well as the intermediate tissue and bloodvessels, are much better displayed.

Preparation 18. Membrane of the fat-cell.—To show this distinctly Ranvier's method may be recommended. An interstitial injection is made with a weak solution of nitrate of silver (1 in 1000), and a minute portion of the fat-lump thus rendered œdematous is removed with scissors, transferred to a slide and covered. Many of the fat-cells exhibit the envelope and nucleus separated by a distinct space from the fat-drop. The silver solution would appear to have penetrated by endosmosis and to have become collected between the fat-drop and its enclosing membrane.

Preparation 19. To complete the study of adipose tissue, the fat-cells should be observed in process of development. For this purpose a preparation of the subcutaneous tissue from a part where fat is being deposited may be obtained from the moderately advanced fœtus of any mammal. The new-born rat is especially to be recommended, since in its subcutaneous tissue there are generally to be found, not only cells which are in every stage of fat-deposition, but others in addition which exhibit the formation of blood-vessels and the simultaneous formation within the same cells of red-blood corpuscles. The mode of preparation is very simple, all that is necessary being to strip the skin from the back, snip off with scissors a little of the gelatinous-looking tissue from the borders of a tract where the fat is already partly deposited, and to place the portion so obtained in a drop of salt solution upon a slide, and cover it with a thin glass.

CHAPTER IV.

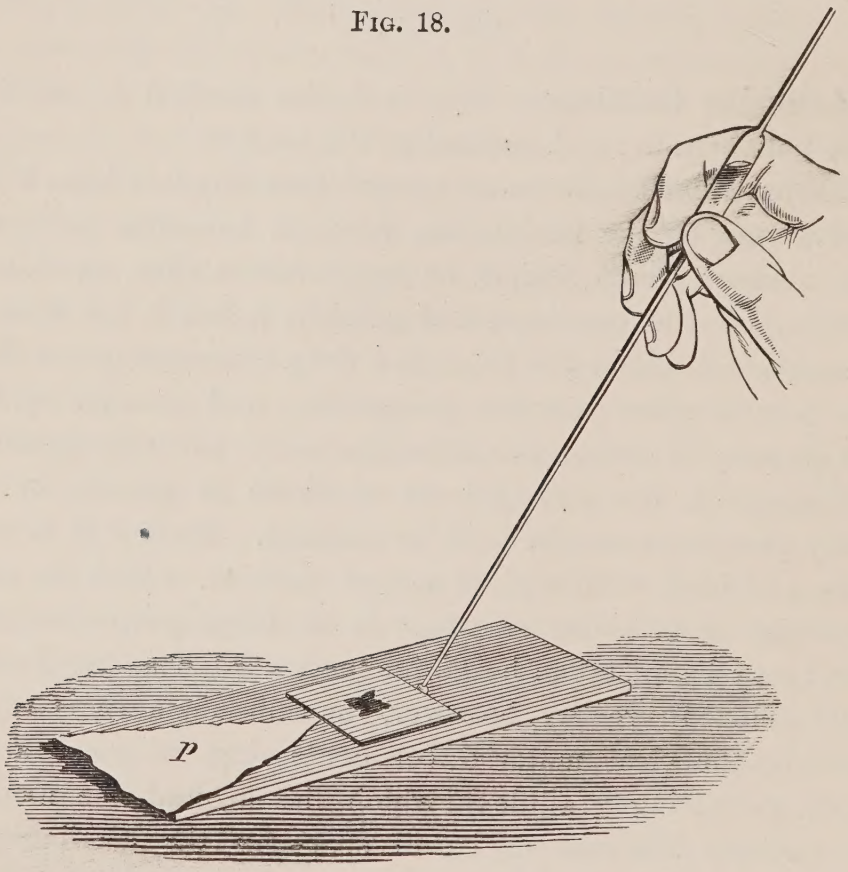
CARTILAGE.

Articular Cartilage.—This is to be studied in sections made both parallel and vertical to the surface.

Preparation 1.—From an animal that has just been killed remove one of the limb-bones, with its articular ends, and with a clean, sharp scalpel or razor take a slice, as thin as possible, off the cartilage, and quickly, before it has time to become dry, transfer the piece to a drop of serum upon a slide, place a cover-glass over the preparation and examine with a high power. Turning the attention more particularly to the cartilage-cells, the arrangement of these in groups in the faintly granulous matrix will be noticed. Each cell is seen to be provided with a clear round nucleus, which in some specimens of articular cartilage is so large proportionately that it may be mistaken by an inexperienced observer for the whole cell. In reality, however, the cell-substance is represented by the clear material (or containing, at most, a few highly refracting granules) which lies around the nucleus and entirely fills the cavity or space in the cartilaginous matrix in which the cell lies. But now replace the serum by distilled water, drawing the former away by means of a piece of blotting-paper placed at one edge of the cover-glass, and allowing a drop of water from a pipette to run under at the opposite edge (see fig. 18), and the picture soon changes. Examine the cells at the borders of the slice, for these are first reached by the water. It will be seen that the clear cell-substance begins to be separated from the matrix, and acquires

a jagged outline, fluid collecting in the interspace which is now left. This clear fluid is stated by some to be water which has permeated the matrix, and passing into the cell space has pushed, as it were, the cell-substance away. But the more probable explanation is that the cartilage-cells, by virtue of a certain amount of vital contractility which they retain, shrink on the application of the water, as they do on the

FIG. 18.



application of many other reagents and on the passage of an electric shock, and that the clear fluid which collects around them is expressed from their protoplasm as it contracts. Whatever the explanation may be, the effect is this : that the now jagged, shrunken cell-body assumes, instead of the clear aspect which in the fresh condition it presented, a coarsely granular appearance, so much so indeed that the nucleus

which was previously so apparent is now entirely obscured. Moreover, as already indicated, the cartilage-cell no longer fills the cell-space in which it lies. The cells always undergo this change after death, unless the tissue has been treated with some reagent which prevents its occurrence.

In preparing a specimen of cartilage with the object of permanently preserving it, our aim should be, as, indeed, with every tissue, to obtain it in a condition and form as nearly as possible approaching that which it had whilst living. There are numerous reagents which, in place of acting like water and causing contraction of the cartilage-cells, fix them in the form they present during life. Amongst these osmic acid may be mentioned first, as the most generally valuable reagent which we possess for this purpose, since it acts in like manner upon nearly all the tissues. But it will not here be recommended for the purpose of preserving the tissue of cartilage in consequence of its costliness, and from the fact that other and cheaper reagents serve the purpose equally well. One special value it certainly has, however; namely, in showing that the little granules in the protoplasm of the cartilage-cell are many of them of a fatty nature, for they are blackened by the reagent. A one per cent. solution of alum, and a saturated solution of picric acid (both recommended by Ranvier), preserve the cells of cartilage admirably. But one of the best and most convenient reagents for the purpose is a weak solution of chromic acid (1 part to 600 of water).

Preparations 2 and 3.—The articular head of one of the long bones is removed from the recently killed animal, split into two down the middle with a strong knife or a chisel, and the halves placed in a large quantity of a solution of chromic acid of the strength above indicated, and allowed to remain in it a few days. The exact time is immaterial, but in specimens, which are left rather longer in the liquid, the bone in the neighbourhood of the cartilage is softened, and the cartilage-cells are more coloured by the acid, and consequently more apparent. When it is desired to prepare the tissue for the

microscope, one of the halves is taken from the fluid, washed in water for a minute or two, and then, the bone being held in the hand, one or two thin sections are taken from the surface of cleavage vertically to the articular surface and extending through the whole depth of the cartilage, including, if possible, a little of the adjacent bone. The sections are placed in water on a slide and covered, and are examined, first with a low power to see the general arrangement of the cartilage cell groups in the superficial, intermediate and deep strata respectively, and subsequently with a high power to see the intimate structure of the cells. These should, as before indicated, present as nearly as possible the same appearance as during life, the only difference being that the tissue generally is less transparent, and slightly coloured, and that the cell-outlines are rather more strongly marked. These differences become even less obvious when glycerine has been permitted to diffuse itself under the cover-glass, for the preservation of the specimen.

Sections are next to be taken parallel to the articular surface and mounted in the same manner; but it must be borne in mind that it is only those sections which include parts of the cartilage near the natural or artificial surface which will be of value as respects the preservation of the tissue elements in their natural condition, at least in the case of the thick articular cartilages of large animals. For the preservative solution naturally takes some time to permeate the cartilaginous matrix, and before it has time to penetrate to the deeper parts the cells will have already shrunk away from the walls of the enclosing cavities and have become changed in the manner previously indicated. So that the deeper sections will exhibit merely the irregular, contracted, and highly refracting corpuscles lying loosely in their cell-spaces.

At the thinnest parts of all the sections cavities may be observed in the matrix which are devoid of cartilage-cells; these having dropped out in the process of preparation.

Preparation 4.—These cavities or cell-spaces of cartilage may be also demonstrated by the same method as was employed to show the cell-spaces of connective tissue, viz., treatment with nitrate of silver and subsequent exposure to the light. For this purpose a fresh joint should be opened, and the articular end of one of the bones (preferably a *convex* one) removed with the saw or bone-forceps. The end thus removed is rinsed in distilled water and then quickly transferred to nitrate of silver solution ($\frac{1}{2}$ per cent.), in which it is allowed to remain three minutes. It is then again rinsed in water, and if necessary gently brushed with a camel-hair pencil to remove adhering silver precipitates; after which it is placed in a beaker of weak spirit and exposed to sunlight. When thoroughly browned, sections are made from the surface with a razor wetted with spirit, and are placed in water (care being taken that they become completely immersed), after which they may be mounted in glycerine.

Some of the sections should be taken from near the edge of the cartilage and mounted on a separate slide. These should show the branched cell-spaces, which present a transition to the much more ramified spaces of the connective tissue of the synovial membrane.

The cells themselves which occupy the spaces are not shown, for, as in silvered tissues generally, the cell-protoplasm remains absolutely unstained; indeed, it is at first sight difficult to believe that the rounded cavities which are seen really contain cartilage-cells. But their nuclei may be brought into view by staining with logwood, subsequently the matrix being coloured by the nitrate of silver. As before mentioned in speaking of the connective tissue, it is, however, difficult, if not impossible, at the same time to bring distinctly into view the outlines of the cell-spaces by the silver method, and the bodies of the cells themselves by some other method of staining.

Preparation 5.—No doubt one of the best and most generally useful methods of colouring cell-protoplasm is that known

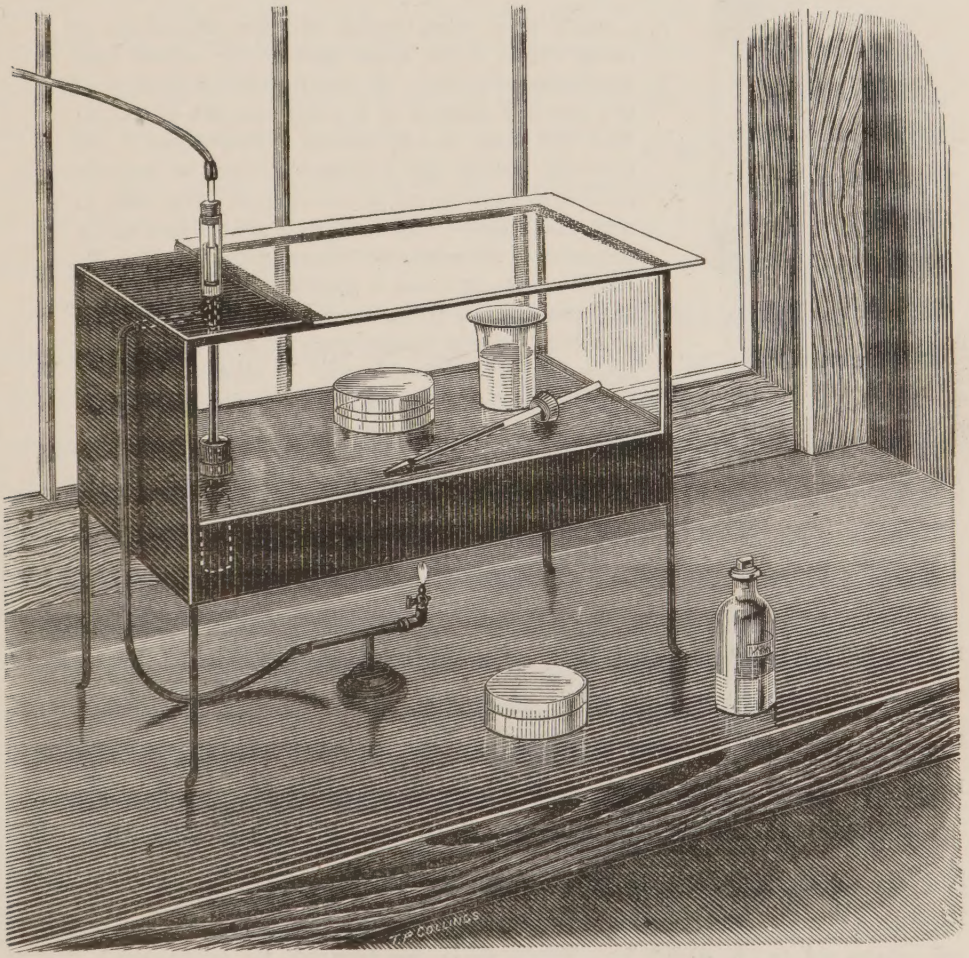
as the gold method. This is applied to cartilage in the following way. Thin sections are made from the articular end of a fresh bone and are placed in a few drops of a solution of chloride of gold (1 part in 200 of water). After half-an-hour they are transferred to a beaker containing a comparatively large amount of distilled water which has previously been slightly acidulated with acetic acid, just so as to be distinctly acid to the taste. The beaker is then covered with a glass plate and placed in a window in as warm a place as possible, and where it will be exposed for some hours a day to the full sunlight. Here the sections are left for two days, after which time they should have acquired a dark violet colour, and are ready to mount in glycerine. But before this is done they should first be examined with a low power in a drop of water to see whether there is any precipitated matter upon the surfaces of the sections. If this is the case it must be brushed away with a camel-hair pencil, the sections being held with fine forceps during the process. When finally mounted and examined with a high power, the cartilage-cells should retain precisely their natural form and appearance, except that they are stained of a faint violet tinge, whilst the matrix remains almost entirely colourless. In sections taken from the edge of the cartilage, the ramified transitional cells which occupy the corresponding cell-spaces shown by the silver method will be rendered apparent.

The colouration by the gold and silver methods appears in some way to depend on the occurrence of a deposition of the metal in those parts of the tissue for which it has the greatest affinity: in the case of gold this is generally the protoplasm of the cells (and the nerve-fibres, where any exist); in the case of silver it is the ground substance, or matrix, or intercellular substance; so that the results of the two processes may, at least in the case of the cartilage-cells, be looked upon as standing to each other in the light of a positive and negative image, using the terms as they are employed in photography.

If it is the winter time, and especially if there is very little bright sunlight, it is of the greatest importance to keep the beaker of acidulated water in which the tissue is placed *warm*, as this materially facilitates

the reduction of the gold. For this purpose it is well to have some arrangement by which the beaker, or several of them, if there be much of the same sort of work going on, can be kept at a temperature of about 30° or 40° Centigrade, and at the same time freely exposed to the light. Fig. 19 represents a convenient apparatus for this purpose. It is made of zinc or tinned iron with loose glass sides and top, and

FIG. 19.

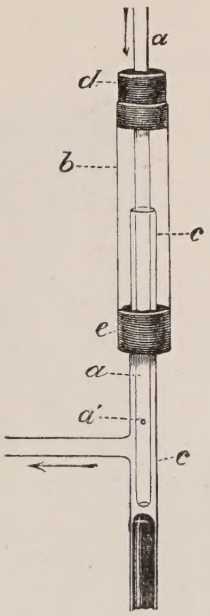


Warming apparatus for maintaining portions of tissue, after treatment by the gold method, at a constant, raised temperature. The lower part of the apparatus is filled with water, into which the bulb of the mercurial regulator dips.

may be fitted with a gas regulator (fig. 20) to prevent the temperature from rising too high. When used for the present purpose the apparatus should of course stand in a good light,—in the window if possible.

Preparations 6 and 7. Costal Cartilage.—The cartilages of the ribs and those of the trachea and larynx may be prepared and examined in a manner similar to that recommended for the study of articular cartilage. Sections, both parallel

FIG. 20.



a, *a*, small inner tube, capable of being slipped up or down within *c*, the upper part of the bulbed tube containing mercury. *a'*, small aperture to allow of the constant passage of a small amount of gas, so that the flame is not entirely extinguished if the mercury should rise sufficiently high to entirely exclude the lower end of the tube, *a*. The tube, *b*, with the corks *d* and *e*, form a simple and easily constructed arrangement (suggested by Mr. F. J. M. Page) which serves as a gas-tight telescopic joint, and enables the tube, *a*, to be raised or lowered according as the temperature of the chamber is desired to be higher or lower. In all other respects the working of the regulator is similar to that belonging to the apparatus shown in fig. 9.

The upper part of the mercurial gas-regulator of the apparatus shown in fig. 19. Natural size.

and vertical to the surface, should be made from the fresh tissue and from pieces which have been a longer or shorter time in chromic acid solution; those from the latter being mounted and preserved in glycerine as before.

Preparation 8.—The alum process may also be advantageously used. Sections as thin as possible, of a perfectly fresh rib cartilage are to be mounted in a dilute solution of alum ($\frac{1}{2}$ per cent.). This will be found to preserve for a time the natural appearance of the cells, better almost than any other reagent.

Preparation 9. Cell Territories of the Matrix.—Sections are to be made of a piece of thyroid cartilage that has been preserved in spirit, and are to be stained with logwood. When sufficiently coloured, they are transferred to water and then mounted in glycerine. The logwood, besides staining the

nuclei of the cells, gives the matrix also a deep purple colour. But this colouration of the matrix is not uniform, for some parts become stained much more deeply than others; those regions more immediately around the cells and cell-groups, and which therefore are, as commonly considered, the latest formed portions, having apparently less affinity for the colouring matter of logwood than the other and older parts. By this method the whole matrix appears marked out into what may be termed *cell-territories*, although series of definite rings can by no means be said to be very apparent around the cell-groups, such as, it is stated, may be produced in sections of cartilage by treating them with a mixture of nitric acid and chlorate of potash.

Preparation 10. Transition to yellow fibro-cartilage.—If the arytenoid cartilage of the ox is sliced longitudinally, it exhibits to the naked eye in its lower part the opaline bluish appearance of hyaline cartilage, but in its upper part the faintly yellow aspect of elastic cartilage, the two parts being separated by a distinct line of demarcation. Take now a thin section from the cut surface, including a little of both parts, and mount, if fresh, in alum solution, or, if the cartilage have been previously hardened in spirit, at once in glycerine. It is immediately seen that the cartilage above the line of demarcation merely differs from that below by the superaddition of a network of comparatively coarse branching (elastic) fibres. Just at the junction of the two parts the fibres are but few in number, and at their ends often imperfect—*i.e.*, they may appear continued merely as rows of granules as if not yet fully formed. Further into the yellowish part they permeate the matrix very thickly, and give it almost a granular appearance, owing to many of them being seen in section. But immediately around each cell there is always a larger or smaller area of the matrix entirely free from fibres, and this area is in appearance exactly like the matrix of hyaline cartilage. So that we may conclude that yellow or elastic cartilage is merely to be regarded as hyaline cartilage, in the

matrix of which a deposit of elastic substance has taken place in the form of branching fibres; and, indeed, the study of its development shows this to be the case.

Preparation 11. Yellow fibro-cartilage.—A tissue may now be studied which is composed entirely of yellow cartilage, the human epiglottis or external ear, for example. A section of either of these shows what at first looks like a granular matrix dotted with islands of hyaline substance, each with one or two cartilage-cells in the centre. The granular appearance of the matrix, when carefully observed, is seen to be due to an excessively close feltwork of fine elastic fibres; in fact, the structure is quite like that of the upper part of the ox's arytenoid, although, owing to their fineness and closeness, the individual fibres cannot be followed for any distance.

Preparation 12. White fibro-cartilage is best studied in sections of any ligament which is composed of this tissue, or of the insertion of any tendon or ligament which is attached, as in the young subject many are, to cartilage. The tissue may have been previously hardened in spirit. Sections should be made both parallel with and across the bundles of tendinous fibres. They are to be stained with logwood and mounted in glycerine. It will be seen in the longitudinal sections that the cartilage-cells lie in chains between the tendon-bundles, occupying the place of the ordinary tendon-cells; from these they are chiefly characterised by their sharpness of outline and (in transverse sections) the absence of lamellar extensions, as well as their greater thickness; but transitions between the two are not unfrequent, especially near the tendon insertion.

CHAPTER V.

BONE.

Preparations 1 and 2. Transverse and longitudinal sections of hard bone.—These are first cut as thin as possible with a fine saw, and are afterwards reduced in thickness and polished by grinding. The bone selected should be thoroughly macerated and bleached; it should be absolutely free from grease. Sections may be made from different bones, flat and long, but for typical specimens of the compact tissue, transverse and longitudinal sections of one of the long bones of the limbs—the ulna, for instance—may be recommended, and a vertical section of one of the flat bones of the skull, such as the parietal, should also be prepared.

The first thing to be done is to get as thin a piece as possible cut with a saw from any desired part. For this purpose a fret-saw may be used, unless a circular saw is available, when this, if fine enough, may be employed with advantage. The piece so obtained is ground down on a hone wetted with water. The hone must have been previously freed from all traces of greasy matter by washing with soap and water with a little soda. The piece of bone is pressed down and rubbed to and fro on the hone simply by the finger, being ground first on one side and then on the other. The feel will be almost sufficient to tell when it is thin enough, and this may be confirmed by placing it on a slide without covering it and examining with a low power. Numerous scratches will doubtless be visible on its surface, produced by the grain of the stone, however fine this may be; but unless

very obvious, they may be disregarded, for they become almost invisible in the subsequent preparation. This consists in well rinsing the piece (when satisfied that it is thin enough) in clean water by aid of a hair pencil, placing it upon a slide to dry, and, when dry, mounting it in Canada balsam. The balsam to be used for this purpose must not be, as is usually the case with that sold in the shops, semi-fluid, but quite hard in the cold—a condition which results from long keeping, or may be produced by heating a little of the more recent resin in a capsule over a sand- or water-bath, until all the volatile matters are driven off. A drop of the melted balsam is then placed upon a slide, which is warmed over a flame until the resin has diffused itself pretty evenly over the central part of the slide. This is then placed on the table, and whilst cooling, but before the balsam has time to become quite hard again, the thin piece of bone is placed upon it. A clean cover-glass is then taken up by the forceps, a drop of the balsam placed upon it also, warmed in like manner, and quickly inverted over the preparation. By this mode of proceeding the balsam fills up and renders invisible the scratches on the surfaces of the section of bone, and some of the Haversian canals may also be filled by it; but it becomes solidified before it has time to penetrate into the lacunæ and canaliculi, which remain, therefore, filled with air, and present the black appearance which is characteristic of any small cavities containing air when they are viewed under the microscope by transmitted light.

Most of the structural points with regard to bone can be seen much better in these preparations of the hard tissue than in sections of a decalcified bone. But it is important in mounting the pieces after they have been ground down to be careful that the balsam does not remain fluid long enough to have time to penetrate into the thickness of the section; if this should happen, the whole is rendered too transparent for any of the details of structure to be made out. On the whole it is perhaps advisable to purchase one or two good specimens

rather than devote a large amount of time to the manufacture of what may after all turn out to be but an indifferent preparation. Sections of hard bone and of teeth are amongst the very few histological preparations which are usually better made by those who make a business of preparing microscopic specimens for sale than by the student himself.

Preparation 3.—In addition to sections of hard bone which are made by grinding in the manner above described, a portion of a well-macerated bone may be placed in a solution of hydrochloric acid (10 parts of the commercial acid to 100 of water) until all the earthy matter is dissolved; the piece is then steeped in a very weak solution of carbonate of soda to free it from the remains of the acid, and sections may then be made with a razor or sharp scalpel and mounted in glycerine. But these decalcified specimens in no way illustrate the structure of the tissue so well as the others.

Preparation 4.—It is, however, quite another question with regard to bones which have not been submitted to previous maceration. For it is to be remembered that in considering the structure of a bone we have to deal not merely with the bony matter pure and simple and its included cavities, but that there are in addition the soft contents of those cavities—the corpuscles in the lacunæ, the blood-vessels in the Haversian canals, and the marrow in the medullary cavity and in the interspaces of the spongy tissue. In order properly to view these perishable structures, we must employ a reagent which will at the same time decalcify and soften the hard matter and preserve and harden the included soft tissues. Such a reagent is to be found in a solution of chromic acid, but as the decalcifying powers of this are but feeble, the portions of bone placed in it must be as small as possible, and a large proportionate amount of the fluid must be used. For example, if the bone be a long bone about the size of the human metacarpal, a piece not longer than a quarter of an inch should be sawn from it and placed

(suspended by a thread if possible) in a beaker capable of containing some 200 c.c. of the chromic acid solution. If the bone be larger, it is well to split the disk thus sawn off into three or four smaller pieces, since otherwise the decalcification will occupy too long a time. The acid used should be at first very weak (1 in 600); in two days' time this may be changed for a solution of 1 in 400; and this again in another two days for 1 in 200. Beyond this the strength of the acid should not be increased, but the fluid should be renewed every three days at least. In addition to this frequent changing of the liquid, it should, throughout the whole time of softening, be stirred as often as possible: this is of the greatest importance, for every agitation brings fresh portions of acid to attack the earthy matter of the bone. By attention to this particular the time which the pieces take to become thoroughly decalcified may be materially shortened. The completion of the process is ascertained by passing a needle through the middle of the piece employed; if it meets with no gritty obstruction, all the earthy matter is probably got rid of. This will most likely be the case in two or three weeks from the commencement of the operation. Should the pieces of bone, owing to their too great thickness or density, be much longer than this before they are sufficiently soft, the process may be hastened by adding to the chromic acid solution a little nitric acid (2 c.c. to each 100 c.c. of chromic solution). This reagent was not available at the commencement of the operation, because, although it would have more readily dissolved the salts of lime than the chromic acid, yet it would have softened still more the included soft tissues of the bone instead of hardening them like that reagent; but it may be employed during the later stages, especially in conjunction with the chromic acid, since the latter reagent has, by its peculiar coagulant action upon the animal tissues, so altered their constitution that their structure is no longer rendered indistinct by diluted nitric, acetic, and the ordinary acids, which produce swelling and

maceration of the *fresh* tissues, and especially of the connective tissue.

The process of decalcification being completed, the pieces are placed in water for a few hours to get rid of the excess of chromic acid imbibed, and are then transferred to spirit; or sections may be made at once without placing the piece in spirit at all. In cutting them, if the bit of bone is too small to be held in the fingers, it may be placed in a split piece of cork. The sections should be very thin, but it is not necessary that they should be large or include the whole thickness of the bone; they are to be stained in the logwood solution, washed in water, and finally mounted in glycerine. The corpuscles within the lacunæ, or at all events their nuclei, are beautifully shown, and all the soft parts are more or less stained, but the actual substance of the bone is very slightly coloured. At the same time, any lines of demarcation which may be present indicating successive deposits of osseous matter in the formation of the bone, or absorption at any part and subsequent redeposition, with the characteristic scolloped edge which such a junction almost always possesses, are very clearly shown by a difference in the colouration. Moreover, if in the section there happens to be any portion remaining of the (ossified) matrix of the original embryonic cartilage, this, like cartilage matrix generally, is intensely stained by the logwood.

Preparation 5.—Instead of chromic, picric acid may be used for the decalcification. A saturated solution of the acid is employed, and care is to be taken constantly to supply fresh crystals of picric acid to take the place of that which is used up in dissolving the lime salts. In all other respects the proceeding is much the same as for the chromic acid method, except that the pieces will require much longer washing in water, and that the spirit in which they are placed be changed several times before the excess of picric acid can be got rid of. The sections may be stained with logwood and mounted in

glycerine in a similar manner, and will show the structure as well, if not better, than those prepared with chromic acid.

Preparation 6. Lamellæ and Sharpey's perforating fibres.—For showing these structures, macerated bones that have been decalcified by hydrochloric acid serve very well. They should before use soak in water for some time, and subsequently lie in spirit, so as to get rid of the last traces of the acid. The point of one of the blades of a sharp pair of forceps is then inserted obliquely into the bone at its outer surface, and a small piece is gripped by the other blade and dragged off in such a manner that it pulls away with it a very thin strip from the superficies of the bone. A few such strips having been obtained, either from different parts of the bone or from the same place (at different depths, therefore), they are placed in water on a slide with the inner surface uppermost and examined with a low power. In some places tapering fibres will be seen projecting from the surface of the torn-off strip like nails projecting from a board; in other parts, round or ovalish holes, corresponding generally in size with these fibres, will be apparent: these are apertures in the lamellæ where the latter have been pierced by the fibres of Sharpey, but those fibres have been pulled out in tearing off the strips. Further, there may be made out a faint appearance of decussation in the lamellæ, like the checks on a plaid, but oblique in direction, best marked near the thin edge of the strip of bone. These appearances are all more evident when the preparation is covered and examined with a higher power. To preserve the preparation it is not a good plan to replace the water in which the strips are mounted by glycerine, since this renders the texture too transparent and the outlines too indefinite. But the strips may be mounted and preserved for a considerable time in water merely if the edges of the cover-glass are fixed first by melted paraffin, and then by layer after layer of chloroform balsam, so as to obviate as much as may be the risk of evaporation.

The bones in which the fibres of Sharpey may best be demonstrated in the way above described are the flat bones of the skull. They

are also to be seen in similar preparations, and in sections of the long bones. If a section be made of such a bone at the place of insertion into it of a tendon or ligament, and in the direction of the fibres of the tendon or ligament, it will be seen that the bundles of fibres of the last-named structures are continued into the substance of the bone as perforating fibres or fibres of Sharpey, so that these in fact almost compose the whole of the osseous tissue at this place (Ranvier). This shows, moreover, that the fibres of Sharpey are to be regarded as bundles of fibrous tissue (connected either with the periosteum or with a tendon or ligament) which were intercalated with the osseous substance proper when this was formed and have become ossified at the same time. When tendons undergo ossification, the bony substance which is formed is wholly of the same nature as the fibres of Sharpey; this may be characteristically seen in the ossified tendons which are met with in the legs of birds.

Preparation 7. Development of bone in membrane.—For the study of the intra-membranous process of ossification it is best to employ the flat bones of the skull of sheep's embryos from two to three inches long. The embryos may have been preserved in Müller's fluid,[1] or spirit, or they may be employed fresh. A piece (corresponding in position with the future parietal, for example) of the still membranous skull-cap is cut out with fine scissors, placed under water if from Müller's fluid, under salt solution if recent, and the skin and muscular layers are torn away from the outside and the dura mater and cartilaginous layer (which in these animals rises up laterally from the cartilaginous basis cranii) from the inside. The membrane in which the bone is being formed is then left. It is held upon a glass slide with a needle or fine forceps, and brushed firmly with a camel-hair pencil, the hairs of which have been cut off short so as to render the stump stiff and resisting. The piece must be kept wet, and examined from time to time with a low power to see whether the edges of the newly-formed bone are sufficiently clear of the membrane and corpuscles, so that the osseous spicules

[1] A solution containing $2\frac{1}{2}$ parts bichromate of potash, and 1 part sulphate of soda, to 100 of water.

and their fibrous prolongations are readily seen. When this is the case the piece is held with the forceps and well rinsed in water or salt solution to free it from loose particles of the soft tissue; it is then placed in logwood solution, and when sufficiently coloured (two or three minutes) is mounted in glycerine. The osteoblasts will be found stained by the logwood; the ossified part is dark and highly refracting, the osteogenic fibres by which it is prolonged remain clear and colourless.

The process of intra-membranous development may also be very advantageously studied in sections of the lower jaw of the fœtus or young animal, as will be afterwards pointed out in describing the mode of preparing this to show the development of other structures, the teeth and hair, for example.

Preparation 8. Development of bone in cartilage.—To study the mode of ossification in cartilage it is not necessary to have recourse to the bones of a fœtal animal, since any which are still in process of growth will serve the same end, and it is more convenient for purposes of manipulation if they have attained a certain size. The long bones of a new-born kitten may advantageously be employed, and the best preparations are certainly those obtained from the recently killed animal, although for permanent preservation it is better that they should have been some days in Müller's fluid. The mode of preparing the *fresh* specimens is as follows. One of the long bones—the femur, for instance—is removed, and its cartilaginous head having been cleared of the adherent soft structures, is split down the middle with a strong scalpel, the split extending a little distance into the subjacent bone. Then by a movement of the scalpel one of the halves is broken away, and the junction of the bone and cartilage is brought clearly into view. One or two slices as thin as possible are now taken from the surface thus produced, including the line of advancing ossification, and are placed on a slide in salt solution and covered. These sections are of course parallel

with the long axis of the bone; but to complete the observation others should be made across that axis, at and a little below the level of the line of ossification, the cartilaginous head being first sliced gradually away until the line of ossification is reached, and then a series of sections taken of the part of the cartilage which is undergoing ossification and of the newly-formed bone.

The fresh preparations are by far the most beautiful and instructive if the sections are obtained sufficiently thin. If it is wished permanently to preserve a section made in this way, the salt solution in which it is mounted must be drawn off by filtering paper placed at one edge of the cover-glass, and at the same time replaced by a solution of osmic acid (one per cent.) applied at the opposite edge. The preparation may then be left for an hour (the filter paper being removed), after which time the osmic acid is in its turn withdrawn and replaced by water, and finally a drop of glycerine is put at the edge of the covering-glass and suffered to take the place of the water as this evaporates.

Preparation 9.—Sections, longitudinal and transverse, of ossifying bones which have been in Müller's fluid for a few days, are prepared in exactly the same manner, except that the treatment with osmic acid is here unnecessary. The preparations can be mounted at once in glycerine.

Preparation 10.—To trace the later steps of the ossification, it is requisite to obtain sections extending rather more deeply into the newly-formed bone than is possible whilst this still retains its earthy salts. For this purpose, therefore, the bone, which may with advantage be considerably smaller than that of the new-born kitten—one from a small fœtus, for example—is decalcified in chromic or picric acid in the same way as was employed for the decalcification of pieces of the fully-developed bone (Preps. 4 and 5). But if the fœtal bone is small, the time necessary for such decalcification will be very much shorter than was requisite for the dense adult bone. In order to cut a longitudinal section sufficiently extensive and thin, it will be necessary to embed the bone in the wax mass (chap. x.), and the sections obtained may be stained with logwood, or successively with both

logwood and carmine, and mounted in dammar varnish in the way usually employed for treating sections. A still better plan is to stain the bone entire with an alcoholic solution of magenta, allow it to become permeated first with oil of cloves and then with melted cacao-butter, and afterwards embed in cacao-butter, treating the sections with warm oil of cloves to dissolve out the fat, and then mounting in dammar varnish. Or the stained bone may be permeated with gum, placed in spirit containing one-sixth its volume of water to harden the gum within the tissue, embedded in wax mass, cut, and the sections placed in water to dissolve out the gum. The object of allowing a tissue of this nature, which consists of hard and soft parts intermingled, to become permeated by the substances above recommended, is that the soft parts and cavities may be filled up by a hard material, so that the tissue may offer a uniform resistance to the razor and the parts of a section may cohere; otherwise the soft parts are very apt to become separated from the hard.

But since these processes are somewhat complicated, the student is recommended to defer them for a while until the methods upon which they depend have been described and explained.

Preparation 11. Medullary tissue.—Ordinary marrow, which consists for the most part of adipose tissue, is obtained from the long bones of most animals. But the spongy tissue of the bones generally and the medullary canal of the long bones in some animals—the rabbit and guinea-pig, for example—are filled with *red* marrow, which contains little adipose tissue, but is mainly made up of the so-called proper marrow-cells, which are in many respects similar to the pale corpuscles of the blood, and, like these, exhibit amœboid movements. To see the marrow-cells, therefore, in their natural condition the tissue should be taken quite fresh and examined on the warm stage. The bone—the femur of a guinea-pig, for instance, preferably a young animal—having been removed and cleared of the surrounding soft parts, is broken across, and a small piece of the marrow picked out and broken up with needles in a drop of serum or salt solution on a piece of thin glass. A small piece of hair is then added, and the preparation is covered; a brush dipped in oil is drawn round the edge of the cover-glass to prevent evaporation, and the specimen is then

placed on the warm stage (p. 18) and examined. The different sizes and varying forms of the marrow-cells are to be noted, as also the large clear nucleus which many of them possess, and which at once distinguishes these cells from the ordinary white blood-corpuscles. But many are also observable which are in every respect similar to the latter, and may indeed not improbably belong to the blood, for the blood-vessels are very large and numerous in the medullary tissue, and yield the numerous red blood-corpuscles which are seen scattered over the field. Transitions may be observed between the proper marrow-cells and the white corpuscles, and as they both exhibit amœboid movements it may be inferred that they are essentially of the same nature, if indeed the one is not to be regarded as derived from the other.

Other cells may perhaps be met with much fewer in number, flattened in form, and sometimes branched, with a large clear oval nucleus, and in some instances containing yellowish red pigment granules. They are larger than the proper marrow-cells, and exhibit no changes of form. They appear to be connective tissue-corpuscles. There is yet another element to be found in the marrow—most likely to be met with in a bit taken from near the inner surface of the bone. This is the myeloplaque or ostoclast, and is characterised by its enormous size—whence the name giant-cell—by its granular appearance, and by containing a number of clear round or oval nuclei grouped together in the middle of the cell, or, in some cases, a single large irregular nucleus with numerous buds from its circumference.

Preparation 12.—To isolate these various elements of the marrow better than can be done in the fresh condition, a piece of the tissue is to be placed in weak spirit (ordinary methylated spirit diluted with twice its bulk of water) for a day or two. After this time a small portion is thoroughly broken up with needles in a drop of water on a slide, is then covered, and stained by allowing dilute logwood solution to flow in under the edge of the cover-glass. The logwood is replaced in a

minute or two by water, and this again by glycerine. By this means a permanent preparation is obtained, which can be studied at leisure, and which very well exhibits the different kinds of cells above enumerated, whilst the red blood-corpuscles are rendered almost completely invisible.

CHAPTER VI.

MUSCULAR TISSUE.

Preparation 1. Involuntary muscle.—It is very easy to isolate the lanceolate cells of which this tissue consists. For this purpose all that is necessary is to place a piece of any organ containing plain muscular tissue—the intestine, for instance—in a weak solution of bichromate of potash (one part to 800 of water) for 48 hours. At the end of this period of maceration, a small strip of either the longitudinal or the circular muscular fibres is torn off with the forceps, placed in a drop of water on a slide, and separated as finely as possible with needles. A cover-glass is then laid on, and the preparation is carefully examined with a high power. The ends and edges of the larger pieces of the tissue have a somewhat ragged aspect, due to the projection from them of the tapering ends of the fibre-cells. In addition to these partially separated elements, others are to be met with scattered over the preparation which are wholly free, and in which all the characteristic appearances of this tissue can be distinctly made out. The elongated nucleus in the middle of each riband-shaped cell can be seen in those cells which lie flat, but it is at present rather indistinct. It may, however, be brought more clearly into view, as can also the faint longitudinal striation which the cells exhibit, by employing one of the smaller holes in the diaphragm of the microscope to admit the light to the object. But to show clearly the nuclei of the plain muscular fibre-cells, nothing is better adapted than staining the tissue with

a weak solution of logwood-alum. It must be used quite dilute, and suffered gradually to diffuse itself under the cover-glass from a small drop placed at one edge. It is not a good plan to draw it through by means of blotting-paper, since in this way many of the isolated elements of the tissue will be drawn away at the same time. But after the drop of logwood solution has passed in great measure or entirely under the cover-glass, a small drop of strong glycerine may be added at the same spot as the logwood. This as it diffuses under the cover will gradually push, as it were, the logwood solution before it, so as to cause the staining fluid to traverse successively every part of the preparation, and eventually to become collected entirely at the opposite edge, the water meanwhile evaporating and leaving the glycerine in possession of the field. All that is needed to complete the preparation is, in the course of a day or two, to fix the cover-glass by painting a little chloroform balsam around the edges.

The involuntary muscular fibres will be seen in section in preparations of the stomach and intestine and numerous other organs, so that it is not necessary to make special preparations at this stage for the purpose. It may, however, be instructive to demonstrate the manner in which the cells are applied edge to edge in order to make up the bundles and lamellæ of the tissue. This is often shown sufficiently well in a thin strip which has been stained with logwood and mounted in glycerine without teasing.

Preparation 2.—But the best preparations for exhibiting the arrangement of the cells are those stained with nitrate of silver. As in other tissues, this reagent stains only the intercellular substance, leaving the cells themselves uncoloured; their outlines are thus brought very distinctly into view. The way to prepare the muscular coat of the intestine by this method is as follows:—A piece of intestine is removed from the recently-killed animal (the large intestine of the frog answers very well for the purpose), and one end being tied up, a glass cannula is fastened into the other end, and the piece of

intestine having been distended with air, this also is closed by a ligature and the cannula removed. The outer surface is then brushed vigorously with a camel-hair pencil wetted with distilled water to remove the epithelioid cells of the serous membrane; a few drops of a solution of nitrate of silver ($\frac{1}{2}$ per cent.) are allowed to flow over the brushed surface and to remain in contact with it for two minutes, the silver solution is then washed off by a stream of distilled water, and finally the distended intestine is immersed in a mixture of equal parts of water and spirit in a beaker and exposed to the light. The immersion is effected either by tying a piece of glass rod or other heavy substance to the gut, or by filling the beaker to the brim and placing a glass plate over it so as to press the piece of intestine under the surface of the fluid. In about an hour's time the preparation may be removed from the light (a few minutes will be sufficient if it is bright sunlight), and a small piece is then to be cut out and mounted in glycerine with the outer surface uppermost.

Preparation 3. Voluntary muscular tissue: cross-striped muscle.—For the examination of this tissue in mammals the animal from which the preparation is to be taken should have been killed some hours previously, in order to obviate the shrinking and contraction which would otherwise take place in a freshly-severed portion of muscle placed in fluid, and would obscure many of the structural appearances. A small longitudinal shred is torn or stripped off from any muscle of the limbs or trunk, placed in a drop of serum upon a slide, and the fibres are slowly and carefully separated from one another, one by one, for as great a length as possible. A very small slip of blotting-paper is then placed by the side of the preparation to avert the pressure of the cover-glass (a hair is not, in this instance, thick enough for the purpose), and the latter is then laid on, and the preparation examined first with a low power, to make out the shape and extent of the fibres, and afterwards very carefully with an ordinary high power, and eventually with as high a power as

it is possible to obtain. In this preparation almost all that is known of the structure of mammalian muscle may be made out—the delicate sarcolemma with the muscle nuclei immediately beneath it, which look clear and oval when the upper surface of a fibre is exactly focussed, fusiform when seen at the edge, the dark cross-stripes of the muscular substance, seen by careful observation with a very high power to be pervaded by parallel rod-shaped particles, the clear stripes bisected by an intermediate dotted line, and finally a longitudinal striation throughout the fibre, which is the better seen in proportion as the transverse striping is less marked.

Preparation 4.—For the purpose of bringing the muscle-nuclei more distinctly into view, another piece may be prepared in a drop of dilute acetic acid (one part in 200 of water). The acid swells up and softens the interstitial connective tissue, so that the fibres are more readily separated the one from the other, and it is found that, whereas in the former preparation the muscle-nuclei could only be made out by exercising the greatest care and attention, they are now extremely obvious, studding the fibres at intervals, but all of them quite at the surface of the muscle under the sarcolemma.

Preparation 5.—When the frog's muscles are prepared in like manner with acetic acid, the nuclei are, on the contrary, seen to be embedded here and there in the thickness of the fibre also.

Preparation 6.—The sarcolemma is extremely delicate in mammalian muscle, and although it may with care be made out even in the fresh preparation, is nevertheless much more easily demonstrated in the muscles of the lower vertebrata, the frog, for example. With this end a piece of the muscle is teased in a drop of salt solution. This fluid has the effect of gradually disintegrating the proper substance of the muscle, so that the latter tends to break down into a clear fluid with numerous fine granules suspended in it, and exhibiting an active Brownian movement. This process of disintegration generally begins at places where the fibres

have been touched by the needles in the process of separation, and if the muscle be fresh the contractile substance breaks and shrinks away at these places, leaving the clear sarcolemma bridging across the interval.

Preparations 7 and 8. Disks and Fibrils.—In order to exhibit the manner in which muscular tissue tends to break up into either disks or fibrils according to the nature of the reagent to the action of which it is submitted, two pieces of muscle are taken from an animal that has been dead some hours, and are placed for a week, the one in a solution of hydrochloric acid (1 in 50), the other in a solution of chromic acid (1 in 200). Small portions are then broken up as finely as possible with needles upon separate glass slides. The fibres from the hydrochloric acid are, many of them, found to cleave into transverse clear disks, some of which will be noticed lying flat, others seen edgeways; whereas in those from the chromic acid there is no tendency whatever to form such disks, but on the contrary, the muscular fibres tend to break up into smaller and smaller longitudinal fibrils.

Preparation 9.—In order the better to compare the fibres either of the same or of different muscles as regards length and diameter, and to see their general shape, it is necessary to isolate a number of them in their whole length. For this purpose the process of separation by the aid of needles is somewhat tedious, and we must turn to reagents which will dissolve the intermediate connective tissue which binds the fibres together, whilst maintaining them intact. Such a reagent is to be found in a solution of sulphurous acid (the liquor acidi sulphurosi of the Pharmacopœia answers very well). The muscle is placed for a week or more in a well-stoppered bottle containing a considerable quantity of the acid, and is kept in a warm chamber (like that shown in fig. 19), heated to about 30° or 40° Centigrade. This very much facilitates the process of maceration, so that after the time stated a mere gentle shaking of the bottle is sufficient to cause the muscle to break up in great measure into its constituent fibres. Some

of these may then be removed, placed side by side in water or weak glycerine on a slide, and covered, with the usual precautions to obviate the pressure of the cover-glass. It will be found that the muscular substance has acquired, in consequence of the maceration, a granular aspect, and that the usual structural appearances are for the most part indistinct. But this mode of preparation may be advantageously employed for the purposes of measurement and comparison of size and form of fibres from different regions of the body.

Preparation 10. Examination of muscular tissue in the living condition. Ultimate structure of a muscular fibre.— The delicacy of the structural elements of the striated muscular fibres of the vertebrata, and the difficulty of maintaining the fibres, when sufficiently isolated, in the living condition, have baffled all attempts that have hitherto been made to satisfactorily determine their ultimate structure before it has become altered either by the death of the tissue or the action of reagents. But the muscular tissue of some of the invertebrata, particularly of insects and crustacea, bears an exact resemblance to the transversely striped contractile tissue of the higher animals, while at the same time the appearances are better marked, and separated portions of the muscle are readily prepared, and may be examined by the highest powers of the microscope whilst still living and freely contractile.

The most convenient of these animals to employ for the purpose is the common great water-beetle (*Dytiscus marginalis*), and the muscles of the limbs, especially the forelegs of the males [1], which are strong and well-developed, should be chosen. A slide and cover-glass having been cleaned, and a lance-pointed needle or a scalpel and fine scissors and forceps being at hand, one of these limbs is cut off at the joint nearest the body, wiped with blotting-paper to free it from the acrid, strongly-smelling secretion which the insect

[1] The males are readily distinguished from the females by their smooth wing-covers, whereas those of the females are ridged and furrowed longitudinally, as well as by their short strong fore-limbs with sucker-like extremities.

emits, and the chitinous integument of the limb is then slit longitudinally so as to expose the pale soft-looking muscle within. Then, the member being firmly held on to the table with forceps or the fingers, as much as possible of this muscular tissue is gouged out with the lance-headed needle or scalpel-point, and transferred to the slide. The muscular fibres are quickly separated somewhat from one another by needles, desiccation being prevented by breathing once or twice upon the preparation, and the cover-glass is immediately laid on *without the addition of any fluid* other than that which naturally moistens the tissue. The preparation is to be examined at once with the highest available power. It is difficult to make out the details of the structure with a combination magnifying less than 1000 diameters, and the defining power must be of the best.

If the object has been quickly enough prepared, numerous fibres will be found which show the successive series of minute muscle-rods, corresponding in position with the darker cross stripes of the muscle, and prolonged into the clear stripes, where they end with knobbed extremities, the juxtaposition of which in rows side by side gives the semblance of a dotted line in each clear stripe, single or double according as the fibre is more or less extended. Other fibres may be seen in which the dotted line is absent, the muscle rods not being enlarged at their extremities; and these being placed end to end in the successive series give an appearance of long, jointed fibrils extending in the direction of the length of the fibre. In such fibres the cross-striped appearance is almost entirely lost, for there are no intervening clear striæ, the appearance of which in the other fibres is probably due to the presence of the rows of strongly refracting dots.

That the bright appearance of the clear striæ may be produced by and dependent on the rows of minute globular ends of the muscle rods is shown by the following experiment:—A drop of water is placed on a slide, and a very small drop of oil is transferred to it by a needle-point, and is then thoroughly whipped up with it, so as to reduce the

oil to as minute globules as possible. The preparation is then covered and examined with the same magnifying power as was used for the water-beetle muscle. The smallest of the oil-globules, like the dots in the muscle, look like mere dark specks, and it will be noticed that each is surrounded by a bright area, an effect always produced by highly refracting bodies when examined in a less refracting medium. And if we imagine a number of such little oil-specks to be placed in a row, there would be a bright band on either side of the row, very like that which is observable on either side of the rows of dots in the water-beetle's muscle.

Contraction of muscle.—In the perfectly fresh preparations of the insect's muscular tissue, spontaneous waves of contraction may be seen passing from end to end of many of the muscular fibres, and a general idea of the phenomena which accompany the contraction, such as thickening of the part of the fibre at the time the wave is passing, and approximation of the cross-striæ, may be obtained. But the contraction proceeds too quickly for the details of the process to be watched, and the fibres are not sufficiently isolated; moreover, it is impossible to say of the particular fibre under examination whether a contractile wave is about to pass along it or not.

Preparation 11.—In order to get the fibres more under command, the preparation must be made rather differently. For the purpose of inducing the muscular tissue under examination to contract at any given moment, a gas-chamber (see fig. 11) is made use of, and a trace of the vapour of alcohol is made to pass over the preparation at the moment when it is wished to observe the contraction of a particular fibre. The mode of obtaining and preparing the tissue is the same as before, except that the piece scooped out of the freshly-excised limb is placed upon a cover-glass instead of on a slide, and the fibres are rather more freely separated. The cover-glass is then inverted over the putty ring of the gas-chamber, at the bottom of which a drop of water has as usual been previously placed. The preparation is searched for a portion of a muscular fibre which happens to be conveniently isolated, and which shows well the normal structure of the living tissue. This having been brought under observation with the highest magnifying power, a little air charged with the vapour of rectified spirit which has been previously poured into the vapour-bottle is blown into the gas-chamber; as soon as the contraction which results is over, the vapour must be replaced by air again, so that the vitality of the tissue is not too soon destroyed. If the preparation is very fresh, the contraction is generally so sudden that it is impossible to follow the

petails of the process; but after a time the tissue responds less actively to the stimulus, and then with care it is possible to make out the changes which are happening in the form of the muscle rods, and in the consequent relative arrangement of the cross stripes of the fibre. Numerous trials, if necessary on fresh preparations, may have to be made before a successful result is arrived at; for various circumstances, especially the shifting of the fibres during their contraction, may tend to vitiate the observation.

Preparation 12.—Examination of muscular tissue by polarised light.—The *polarising microscope* is nothing else than the ordinary microscope with the addition of two Nicol's prisms, one placed below the object and another above the ocular; the upper one is generally mounted in combination with a low ocular, so that it is not necessary to use the ordinary eye-piece. The light, coming from the mirror, becomes polarised as it passes through the lower Nicol (the polariser). If now the upper Nicol (the analyser) be slowly turned round as it is being looked through, it will be found that there are two positions in which the field is quite dark; that is to say, the polarised rays are entirely cut off. By observing now the relations of the prisms at these positions of total darkness, it will be found that their planes of polarisation—as shown by the way in which the prisms are cut—are at right angles to one another. In all intermediate positions a greater or less amount of light is enabled to traverse the analyser. But if any object which possesses the property of refracting light doubly is placed upon the stage of the microscope, and examined, and if then the field is made dark by turning the analyser, it will be found that the doubly refracting substance remains bright, unless it happen so to lie that its optic axis is parallel with the plane of polarisation of either Nicol. And if the object be a muscular fibre at rest, the whole fibre will appear bright and doubly refracting, whereas if it be in the state of contraction the bright stripes only will allow the light to pass, the dark stripes in this condition of the fibre being singly refracting.

These observations are best made upon the living muscle of the water-beetle by aid of the gas-chamber. The portion of fibre under observation should be quite free and not overlaid by other fibres. The change in the optical condition of the fibres which ensues on contraction may, if due care and patience be exercised, in this way be made out. The results arrived at by the examination of portions of the tissue which have been hardened in alcohol, and mounted in glycerine or Canada balsam, although more easily seen, are less trust

worthy, since the muscular fibres are liable to undergo considerable changes after death, and under the action of re-agents.

A pretty modification may be made by substituting a thin piece of mica for the covering glass. This causes the field of view to become tinted, the particular colour varying with the thickness of the mica, and the relative position of its optic axis to those of the Nicols, and any doubly refracting substance which is now examined assumes the colour which is complementary to that of the field. The object of the revolving stages with which the larger microscopes are generally fitted is to enable the observer to modify the position of the optic axis of the tissue which is being examined, with relation to those of the Nicols; and it also serves when the mica is used to change in like manner the relative position of the optic axis of this also, and thus to modify the colour of the field of view.

Transversely striated muscle is not by any means the only tissue which is doubly refracting, for the property is possessed by the white fibrils of connective tissue, and by bone, as well as by the plain muscular fibre-cells. But it is the only one which under certain conditions exhibits alternate bands of singly and of doubly refracting substance. It has, however, been pointed out by Ranvier that it is rather the conditions of growth and formation of a tissue than differences of structure which tend to determine differences in the optical properties of the substance of which it may be composed. And he instances the case of cartilage, the matrix of which, although undoubtedly composed of the same substance throughout, is doubly refracting in those parts where the cells, either from pressure or in progress of growth, have come to assume either a flattened or elongated shape, singly refracting where they remain rounded.

Preparation 13. Transverse section of muscle.—In examining the fresh muscle of the water-beetle, in either of the ways above described, it may happen that a fibre is seen which is turned up towards the observer, and in which, therefore, by focussing the vertical part with the fine adjustment, a view may be obtained just as if the fibre had been cut transversely. It is then said to be seen in optical section; and for muscular tissue this is the only way in which a section of the fibres, whilst they are quite unaltered, can be observed. Nevertheless, when it is not so much the structure

of the fibres but their shape and mode of arrangement into bundles that is to be the subject of investigation, the tissue may be hardened by weak chromic acid ($\frac{1}{2}$ per cent.), or by alcohol, and thin transverse sections may be cut with a razor and mounted in glycerine, with or without previously staining in logwood. There is another method, that of freezing, which has been much recommended for hardening fresh muscular tissue in order to obtain transverse sections of the fibres in what has been thought a completely unaltered state. It may be doubted, though, whether the muscular substance does not undergo some structural alteration either in the freezing or in the subsequent thawing. Yet, as it is frequently employed for this tissue, and is of considerable value in special investigations into the structure of other tissues and organs, the method may be here described.

Preparation 14. Section of frozen muscle.—The best and simplest mode of freezing a tissue is that recommended by Urban Pritchard. A solid cylinder of copper ($1\frac{1}{4}$ inch wide and 3 inches long), with flat ends, is placed for a few minutes in a freezing mixture composed of equal parts of pounded ice and salt, by which it is cooled to considerably below the freezing-point. The copper cylinder is then removed, wiped quite dry with a cloth, and a piece of flannel is wrapped three or four times round it, leaving one of the flat ends exposed. The muscle, which may conveniently be taken from the leg of a frog, is cut across near its middle, and one of the halves is placed with its cut surface on the flat end of the copper block in a drop of gum arabic solution. This as well as the tissue very speedily becomes frozen throughout, adheres to the copper, and then, the block being held in the left hand, sections are made of the muscle with a dry razor, which should have been previously cooled by contact with the vessel containing the freezing mixture, and the sections are at once transferred to a slide and covered, either in serum, or, better, without the addition of any fluid.

Sections made by freezing possess the advantage that,

since they have not been submitted to any coagulating reagent, they may be treated with nitrate of silver, chloride of gold, and other fluids which are of value only when the tissues are perfectly fresh.

Preparations 15 and 16. Termination of muscle in tendon.—If the tendons of a mouse's tail are forcibly drawn out, after nipping off the end of the tail in the manner described in the account of the preparation of tendon (p. 67), it will generally be found that portions of a number of small muscular fibres are adherent to each tendon, for the fibres have their insertions into the tendon, and are ruptured by the force employed. These ends, mounted in serum, serve conveniently for the study of the mode in which the fibres of a muscle terminate in a tendon, when the fibres of the latter run in the same direction as those of the muscle. But, easy as the tissue is to prepare, the observation is complicated by the fact that the muscular fibres form generally somewhat of a clump as they pass to end in the tendon. The preparation may be improved by being stained with picrocarmine solution. This colours muscular tissue yellow, tendinous tissue red, so that the distinction between the two is made more obvious. It is best to take a few freshly drawn-out tendons, and to mount their ends in a drop of the picrocarmine solution, surrounding the edges of the cover-glass with melted paraffin to prevent evaporation of the liquid.

It is easier to make out the way in which the fibres of a very thin flat muscle, such as the subcutaneous muscle over the frog's sternum, terminate in the fibrous tissue to which the muscle is attached. Here the fibres of the tendon do not take the same direction as the muscular fibres. A frog, having been killed by destroying the brain and spinal cord, is laid upon its back, and the skin over the throat is reflected downwards towards the sternum. The flat muscle which is thereby brought to view passing to the skin is snipped across the middle with scissors, seized on the cutaneous side of the snip with forceps, and a square piece, including its inser-

tion into the skin, is cut out. It is placed as flat as possible upon a dry slide, the curled-up edges being turned down by the aid of mounted needles, and a drop of serum having been placed upon a cover-glass, this is inverted over the preparation. The rounded ends of the muscular fibres are seen fitting into little depressions in the fibrous tissue, and with prolongations from this passing around and between them.

The blood-vessels of muscle will be studied later, after the methods of injecting and of cutting sections have been described. The mode of termination of nerves in voluntary muscle will also be most advantageously deferred until the preparation of the nerves themselves has been treated of.

CHAPTER VII.

NERVOUS TISSUE.

Preparation 1. Medullated nerve-fibres.—For the study of the medullated nerve-fibres a piece of one of the ordinary nerves—those of the limbs, for example—may be cut out from any recently-killed animal. If the nerve be a large one, a thin strip only should be used, preferably taken from the interior after the piece has been torn longitudinally into two halves by fine forceps. The strip is to be placed on a slide in a little serum or salt solution, and carefully separated as finely as possible. This separation must be effected, not by seizing the piece anywhere and tearing it up at random, but by inserting fine needles into it near one end and gently drawing them asunder, so that the piece is split into two. Repeating this process a number of times on the resulting pieces, the nerve will be eventually separated into very fine bundles of fibres, together with a number of more or less isolated fibres, which are still nearly straight and uninjured, except near one end. The preparation may then be covered, and the general character and appearance of the fibres investigated. To see the nodes of Ranvier well, a tolerably large fibre, free for a considerable part of its length, should be chosen, and by moving the slide it should be carefully followed with an ordinary high power. It will be found that at definite and not very close intervals along the fibre the double-contoured medullary sheath fails altogether, and the axis-cylinder alone continues the nerve at these points. It is not very easy to see the oval nucleus in the middle of each segment in the fresh, unstained preparation.

Preparation 2.—Osmic acid possesses the property of staining fatty matters of an inky-black colour. Since the medullary sheath of the nerves is mainly composed of a phosphuretted fat, this becomes accordingly stained by the acid, whilst the other parts are left of a greyish tinge. Moreover, the regular breaks in the fatty sheath—the nodes of Ranvier—are by this means brought into prominence, owing to the complete intermission of the darkly stained medullary substance at those places. At the same time the axis-cylinder can be distinctly made out crossing the interval, and the primitive sheath, or sheath of Schwann, can be often seen both here and also near the centre of each of the segments into which the fibre is divided, where it is bulged out by the presence of the oval nucleus. The method for preparing these osmic preparations is as follows:—From an animal which has been quite recently killed, a small nerve, not larger in diameter than an ordinary thread, is chosen, and a piece about half-an-inch long is cut out, but in doing so care must be taken not to drag upon or injure the nerve more than is absolutely necessary. The piece is placed for four hours in a little covered glass pot containing a drop or two of a 1 per cent. solution of osmic acid; it is then transferred to water for an hour, and finally placed in a mixture of glycerine and water (equal parts). It can either be teased in this at once or left for a day or two or even longer; in preparing it, the same precautions must be used as were recommended for the preparation of the fresh nerve (see preceding paragraph).

Preparation 3. Fibres of Remak.—To see the grey or non-medullated fibres, pieces of the sympathetic nerve are taken from the neck of an animal and prepared and examined both fresh and after treatment with osmic acid, in the same manner as the cerebro-spinal nerves.

Mode of union of the nerve-fibres to form the nervous cords.—In the several teased preparations both of the cerebro-spinal and of the sympathetic nerves there will be seen, especially in those prepared with osmic acid, besides the

actual nerve-fibres, in the first place, a quantity of connective tissue, for the most part of the nature of areolar tissue, which formed a general ensheathment for the nerve, and sent partitions in between its several bundles or funiculi; secondly, the special sheaths of the funiculi, which become torn and stripped away in the process of teasing, and which look like flat bands composed of an almost homogeneous substance, but pervaded by a network of fine elastic fibres and with round or ovalish nuclei scattered upon them here and there. Thirdly, there will be seen running along close to and surrounding the nerve-fibres themselves very delicate, nearly straight fibrils of connective tissue, with here and there the nucleus of a connective tissue corpuscle. These three forms of connective tissue represent respectively the epineurium (cellular sheath); the perineurium (neurilemma); and the endoneurium, or tissue within the funiculus; but their relative situation and arrangement, as well as the lamellated structure of the perineurium, can only be properly displayed by transverse sections of a nervous trunk, the mode of preparing which will not be treated of until the general directions for the preparation of sections have been given.

Preparation 4.—But the cell-outlines on the lamellæ of the perineurium may be shown by the silver method. For this purpose either a very small nerve is chosen and a piece of it is removed and immersed for ten minutes in half per cent. silver nitrate solution, after which it is washed in distilled water and exposed in glycerine to the light; or, should it be wished to prepare a larger nerve consisting of more than one funiculus, this is carefully dissociated in a few drops of the silver solution, and after a like treatment is also to be mounted in glycerine and exposed to the light. After a few minutes' exposure to sunlight the preparations may be examined. It will be found that the sheath of each funiculus or nervous bundle is covered by large epithelioid markings, and if the preparation be very successful, two, three, or even more layers deep of such markings may

be counted by examining with a high power and carefully adjusting the microscope.

In addition to this it will generally be found that the medullated nerve-fibres themselves are marked transversely at each of the nodes of Ranvier by a dark line, or rather ring, surrounding the axis-cylinder. These markings appear to be owing to the existence of a substance here, between the segments of the nerves, which, like the intercellular substance elsewhere, has an affinity for the metal. Finally, at many of the nodes of Ranvier, particularly if the immersion in the silver solution had been rather prolonged, the latter will have penetrated to and become reduced in the substance of the axis-cylinder, which therefore will here have the appearance of a dark line or band piercing the ring of intersegmental substance, the two together having the semblance, under a moderate power, of a little black cross upon the nerve at those points. Sometimes in such preparations the axis-cylinder, where stained at the nodes by the reduced silver, presents a cross-striated appearance, but the meaning of this is not known.

Nerve- and ganglion-cells; structure of the nerve-centres.—To study the exact form and appearance of the tissue elements of the nervous centres they must be as much as possible isolated and examined in teased preparations, although their position and local relations are best made out in sections of the several organs in which they occur. For the present we will confine ourselves to a description of the best methods for isolating the nerve-cells. For this purpose the tissue is treated similarly from whatever part of the brain or spinal cord the piece to be examined is removed; but, as a typical example, a piece of the spinal cord from the lumbar region may be taken, for the nerve-cells are here very numerous and consequently more readily found than in the other regions.

Preparation 5.—The human spinal cord is, if obtainable, best adapted for the study of the cells, for they are readily picked out under the dissecting microscope, in consequence of

the little mass of dark pigment which each contains. If a piece of the human spinal cord cannot be got, the spinal marrow of the ox or sheep, or other tolerably large animal, may be employed. The piece to be macerated should be about half an inch long, and is placed for forty-eight hours in weak bichromate of potash solution (1 in 800). At the expiration of this time it is taken out of the fluid, and with the point of a scalpel or of fine scissors a small piece of the grey matter is dug out from the anterior cornu, placed in a drop of water upon a slide, and separated with needles into three or four pieces. These are then placed under the dissecting microscope and carefully broken up further; those parts where the cells are most numerous being singled out, and the preparation being examined every now and then on the ordinary microscope with a low power, in order that the progress of the isolation may be watched. When as much as possible has been effected in the way of separating the nerve-cells a piece of hair is placed in the fluid and the cover-glass gently superposed. The cells may then be sought with a low power and carefully examined with a higher one.

If the preparation is very successful, and it be desired to preserve it, keeping the cells at the same time as much as possible of their natural aspect, the best plan to adopt is to allow a drop or two of a 1 per cent. solution of osmic acid to flow in at the edge of the cover-glass, and after it has acted upon the tissue for an hour, by which time the cells will have become stained of a dark greyish tint, to carefully run through from the same side first a little distilled water to wash away what remains of the acid, and then glycerine to preserve the preparation, after which the edges of the cover-glass may be cemented. Besides the nerve-cells, which in a carefully-prepared specimen may be very well seen, with their long branching processes extending in some cases far beyond the field of the high power objective, some points in the structure of the nerve-fibres of the spinal cord can be well made out in these preparations. For

example, it may be readily seen that the medullary sheath has broken away in many parts from the white nerve fibres, and where not actually removed has become swollen and coagulated in irregular masses around the axis cylinder, changes which could hardly have taken place were there any structure surrounding the nerve fibres such as the primitive sheath of the peripheral nerves. Further, where the axis-cylinders are in this manner laid bare, as they often are for a considerable part of their length, their fibrillar structure can, with a high power, be made out without difficulty. A similar structure can also be seen in the processes of the nerve-cells, and extending from them through the body of the cell itself.

Preparation 6.—The cells from the ganglia, whether spinal or sympathetic, are isolated in a manner similar to that employed in the case of the spinal cord, except that the period of maceration in the bichromate solution needs to be longer, owing to the much larger amount of connective tissue by which the nervous elements are invested. At the same time it is quite possible to get a certain number of the cells sufficiently isolated, even from the fresh ganglion, without any maceration; for each cell being loosely contained in a special capsule of flattened cells, it readily falls out when the nerve fibres with which it is connected are ruptured. But although the cells themselves of the ganglia are readily enough separated, it is very difficult, both in the fresh and in the macerated preparations, to get their continuation to the nerve fibres, these being left behind for the most part. The mode of permanently preserving the specimen is like that employed in the preceding preparation, except that the cells may with advantage be coloured by logwood instead of osmic acid.

Terminations of the nerves.—The description of the mode of preparing and demonstrating the terminations of nerve fibres in various special parts of the body will be deferred until those parts and organs are severally treated of, but the Pacinian bodies, in which many of the sensory fibres end, and

the end-plates in which the nerves supplying the voluntary muscles terminate, may be now prepared.

Preparation 7.—The **Pacinian bodies** are very readily found in the cat's mesentery. Here they are at once seen when the abdomen is opened and the membrane is held up against the light, as clear, oval specks, either dotted singly here and there or forming groups of two, three, or more. There is generally a considerable group in the meso-rectum, and moreover they are here usually not so much obscured by the adipose tissue as in the mesentery proper. By far the best general idea of their structure and the relation they have to the nerve fibre entering them is obtained from their study in the fresh condition, without the addition of reagents. But it is well to separate them from the surrounding tissue of the mesentery, for this is often loaded with fat, and, when not, the fibrous tissue of the membrane tends to obscure the structure of the little bodies.

In order to isolate one of them cut out the piece of the mesentery containing it, carrying one of the cuts close along the edge of the corpuscle. Then place the excised piece upon a slide in a drop of serum, and without actually transfixing the Pacinian itself, tear away the investing mesenteric tissue bit by bit under the dissecting microscope. It will be found that with a little manipulation the corpuscle shells out as a lemon-shaped body, with a twisted stalk at one end. It is to be examined with a low power to make sure that the fat is entirely removed; the débris may then be wiped away, a little fresh serum added, a narrow slip of blotting-paper placed close to the corpuscle to avert the pressure of the cover-glass, which is then laid on and the specimen examined.

In these fresh preparations, owing to the extreme transparency of the layers of the capsule, the core of the corpuscle with the central fibre, together with the mode of passage of the nerve fibre into this at the stalk, can be made out better than after the action of reagents. And still more so if

the outer layers of the capsule are removed altogether, as may readily be done with fine needles under the dissecting microscope, leaving only the core and the closely-set layers of the capsule which immediately surround it. In tearing away the outer layers it will often happen that the perineural or neurilemmal sheath which surrounds the nerve fibre as this passes into the corpuscle is torn off along with them, since they are directly continuous with it.

These fresh preparations of the Pacinians are very beautiful, but unfortunately they cannot be preserved in that state. Treatment with glycerine causes the corpuscles to shrink and become too transparent, and most of the ordinary staining fluids colour the core too deeply and obscure the termination of the nerve fibre. If it be desired to preserve any such preparation as showing some one or more points particularly well, the cautious employment of osmic acid prior to mounting in glycerine is most to be recommended. The serum in which the corpuscle is mounted must first be replaced by salt solution; this again by 1 per cent. solution of osmic acid, and this after being left for an hour or more in contact with the preparation by distilled water, whilst finally a drop of glycerine is allowed gradually to diffuse in from the edge of the cover-glass.

Preparation 8.—The structure of the tunics which form the lamellated capsule of the Pacinian body is not well shown in the fresh preparation owing to the transparency of the object; indeed, it is the lines of contact between successive coats which look like layers of the capsule; the substance of the coat being clear and pellucid, gives the notion of an intermediate fluid. In order to show their fibrous connective tissue structure (the white fibrils running transversely, and collected for the most part near the surfaces of each tunic, and the elastic fibrils forming a network through the thickness) some of the little bodies should be dissected out in the way above described, and placed for ten days or a fortnight in a $\frac{1}{6}$ per cent. solution or

chromic acid. They are then placed on a slide in a drop of water, and with fine and perfectly clean needles are broken up under the dissecting microscope bit by bit, commencing at one end and breaking off transverse pieces. It will be found that, owing to the direction of the fibrils, the corpuscles tend to break across into disk-like portions. The core does not share this tendency, but this is of little consequence, for it is not well displayed in these preparations. A piece of hair having been added, the preparation is covered and examined. Small fragments will probably be found which give a sectional view of the tunics, and others in which they are seen flat. If a little logwood solution is allowed to run under the cover-glass, nuclei on the surface of the tunics may here and there be stained.

Preparation 9.—These nuclei belong to flattened epithelioid cells, which cover both outer and inner surface of each tunic, and which, seen in profile, are in reality the well-defined lines seen in the fresh Pacinian, and long described as the coats themselves. The outlines of these cells may be brought into view by staining with nitrate of silver. For this purpose, as is always the case with silver-preparations, the tissue must be fresh and unacted on previously by any other reagent. One or two corpuscles are to be thoroughly isolated and freed from surrounding mesenteric tissue and fat; placed for ten minutes in silver solution (1 in 200); washed in distilled water, and exposed in glycerine to the sunlight until of a greyish colour, when they may be covered, and examined.

Preparation 10.—To complete the study of the Pacinians, sections should be made of them, but as they are very small, it will be best to defer this until some practice has been attained in the art of cutting microscopic sections. A convenient way to prepare them, in order to show the various parts to advantage, is as follows:—A very small piece of meso-rectum, containing several corpuscles close together, is cut out (if such can be found; if not, one or more may be isolated as before), and placed in a small beaker containing 100 cub. cent. of a weak solution of acetic acid (1 in 200), to which about

5 c.c. of the ordinary (½ per cent.) chloride of gold solution has been added. The tissue is kept in this, in the light, for three or four days—until it has become of a dark violet colour; it is then placed for a day in weak spirit, and then in strong, and two or three days later is ready for embedding and cutting. In embedding the piece of tissue, it should be so placed that the corpuscles, at least most of them, are cut as nearly as possible transversely.[1]

Preparation 11.—The **end-plates**, or terminal expansions of the motor nerves, are difficult to find, and so soon undergo alteration as speedily to become unrecognisable. On this account it is necessary, in searching for them, to employ only muscles which are absolutely fresh. In mammalia the best muscles to choose for the purpose are those of a lamellar shape and with short fibres, such, for instance, as the intercostals of small animals. The muscular fibres are severed close to their attachments, so as to get them in their whole length, and the small, thin piece of muscular tissue obtained is quickly transferred to a slide, and mounted, either without addition of fluid or in a small drop of serum which is put on the cover-glass before this is inverted over the preparation.

This should now be thoroughly searched with a good immersion objective for the nerve endings. Branches of the intercostal nerve will be found running across the direction of the muscular fibres. Starting from one of these, trace carefully one by one the single nerves which pass off from it. It will be found generally that they branch one or more times, and eventually the resulting twigs pass off to the muscular fibres, each fibre receiving one of the nerve-twigs. They retain their medullary sheath until the muscular fibre to which they are attached is reached, when the sheath suddenly ceases to be visible, and it is by following the single fibres until they come in this way to an abrupt termination, that an end-plate may be met with. But even if the place where the

[1] A description and delineations of the appearances exhibited by the Pacinian corpuscles when prepared in these several ways will be found in the 'Quarterly Microscopical Journal' for April, 1875.

nerve-fibre joins the muscular fibre is arrived at, it is still in most cases difficult to make out the exact mode of termination, in other words, the structure of the plate. The utmost that can generally be seen is a clump of clear, round nuclei embedded in a granular material. The difficulty arises partly from the readiness with which these structures undergo alteration after removal in warm-blooded animals, and partly from the fact that they are often obscured by super- and sub-jacent muscular fibres or bloodvessels.

Preparation 12.—In the common lizard (*Lacerta agilis*) the end-plates may be much more easily found and satisfactorily seen, but still the utmost care must be taken in the preparation. The animal having been decapitated and the trunk pinned out upon a cork, a piece of one of the limb-muscles—including the whole length of the fibres—is removed and placed on a slide in a drop of serum. The fibres are then isolated under the dissecting microscope as carefully and completely as may be, and a piece of paper or a hair having been added to avert pressure on the tissue, it is covered and the fibres are examined with an immersion along their whole length. Of course, if no end-plates can be found in the first specimen another must be taken, but it will generally not be very long before one is found, either at the edge of a fibre, and therefore seen in profile, or on the surface, and seen flat.

Unfortunately, a method has not yet been discovered by which they may be well preserved. That which on the whole answers the best is to treat the preparation with 1 per cent. osmic acid, having first washed away the serum by salt solution. After the osmic has been about an hour in contact with the tissue, it may be washed away and glycerine substituted. In this way the form of the little end-plate or prominence is well preserved, and the sudden termination of the medullary sheath of the nerve fibre well shown, since this becomes blackened by the osmic acid, but the substance of the end-plate becomes dark and granular, so that the nuclei are with difficulty seen.

CHAPTER VIII.

THE BLOODVESSELS.

Preparation 1. The larger bloodvessels.—The **epithelioid lining** can only be properly demonstrated in fresh bloodvessels stained by nitrate of silver. For this purpose a piece of a large vessel—artery or vein—is obtained, either from a recently-killed animal or from an amputated limb, and having been slit open with scissors it is pinned on to a cork with the inner surface uppermost. Care must be taken in doing so not to rub this surface in any way. A little distilled water is then spirted over the preparation from a wash-bottle, with the object of removing any blood or serum that may remain on the wall of the vessel, and a few drops of half per cent. nitrate of silver solution are allowed to flow over the surface. After a minute it is again washed with distilled water, and is then put at once into a beaker of spirit and placed in the sunlight. After a time the surface will have acquired a greyish tinge, with a browner patch here and there; it may now be removed from the window, but should be left in spirit until the next day, when it will be hard enough to enable thin sections to be made with a razor from the inner surface. In order to cut them it will be found convenient to remove the piece of bloodvessel from the cork, and to hold it by one end by the thumb and fingers of the left hand, so that the piece rests by its outer surface on the ball of the finger; the razor is then dipped into spirit, and as thin a slice as possible (it need not be very large) is removed from the inner surface of the bloodvessel and placed in the spirit, after which one

or two more may be taken from other parts. In making sections when the piece is held in this way, it will be found convenient to cut *from* the operator.

The slices are taken up on a needle or section-lifter and placed on a slide with the stained surface uppermost; they should be in as little spirit as possible, but at the same time should not be allowed to become actually dry. A drop of the ordinary glycerine solution is placed on a cover-glass, and this is quickly inverted over the sections. On examining them with the microscope the outlines of the epithelioid cells, and perhaps also their nuclei, will be seen in those sections which were made from the *grey* part of the bloodvessel. In sections, however, which include any of the patches which look *brown* to the naked eye, it will be found that the difference of colour is due to the epithelioid cells having at these parts become accidentally rubbed or washed away before the silver solution was allowed to act; for since the subjacent tissues (the sub-epithelial connective tissue, if present, and the muscular tissue of the middle coat) contain more intercellular or ground-substance than the epithelioid layer (where it only occurs in fine lines between the cells), they assume a browner appearance after the reduction of the silver, and show under the microscope in the one case irregular white patches—the cell spaces—upon the brown ground, and in the other transversely arranged lanceolate white markings—the plain muscular fibre cells—with a variable amount of ground-substance between. The latter appearance may be obtained all over the preparation if the bloodvessel which is to be treated wth silver nitrate is first brushed with a camel-hair pencil moistened with distilled water, for by this means the epithelioid cells are removed, and also, for the most part, the sub-epithelial connective tissue where present, the elastic layer being the only part of the internal coat which remains, and since this does not reduce the silver salt the muscular layer is the one which is seen in such cases. Except when the epithelioid cells are first

removed, either purposely or accidentally, even a comparatively long exposure to the action of the nitrate of silver solution will not cause the deeper coats to become stained. This is the case with all structures which are coated with epithelioid cells.

Preparation 2. Elastic layers; fenestrated membrane; muscular tissue.—To prepare these several parts, a piece of artery (or vein) is taken (as fresh as possible, but this is not so imperative as for the preparation of the epithelioid layer), and placed for a week or more in a weak solution of bichromate of potash (about 1 in 800), the fluid being changed every other day. The piece is then taken out, pinned down on a cork, with the inner surface uppermost, and a thin strip torn off from the inner surface with fine forceps. This is transferred to a slide, and teased as finely as possible in a drop of water. It will be found advantageous to employ only as much water as will keep the tissue moist, and to add more of this by placing a drop on the cover-glass before it is laid on. If the small pieces are then examined, it will be found that they are for the most part made up of a close network of elastic fibres of varying degrees of fineness. Many of them have very broad fibres and small meshes, so that there may be found in different arteries every transition to the true elastic membrane. This will itself in all probability be met with projecting at the edges of some of the fragments of tissue, or even entirely separated; the fragments are generally curled at the edges, are often striated, and nearly always exhibit rounded holes. These fenestrated membranes are more frequently met with in the inner coat of the smaller or medium sized arteries (such as the basilar), than in the largest vessels (such as the aorta).

But besides the different kinds of elastic tissue there is also to be found in nearly every such preparation a number of plain muscular fibre-cells scattered about in the fluid; for, in stripping off the inner coat, shreds of the middle coat nearly always adhere to it, and the muscular cells of this

readily separate after the maceration in bichromate. But the isolated cells present in most instances such a ragged shapeless aspect that they would hardly be known for muscular tissue. A convincing proof, however, is the addition of a little weak logwood solution at the edge of the cover-glass. This, as it comes to each of the cells in question, almost instantaneously stains their long rod-shaped nuclei of an intense violet, whilst the body of the cell, if the logwood solution be sufficiently weak, remains uncoloured. The addition of a little glycerine at the edge of the cover-glass, and the subsequent cementing, are sufficient to preserve the preparation.

The connective tissue and elastic fibres of the outer coat (as well as the muscular tissue of the same coat in certain veins) can be equally well seen in a teased-out preparation.

Preparation 3. Study of the structure of the bloodvessels by means of sections.—To form a correct idea of the relative thickness of the several coats, as well as to observe the differences in arrangement in different arteries and veins, it is necessary to study them in vertical sections, *i.e.*, sections made in a direction at right angles to their surface. Such sections may be either transverse or longitudinal; it will be better perhaps to choose the former direction, for the middle coat is thereby better displayed. But the vessel to be cut must first be hardened. This may be effected speedily by immersing it in very strong spirit for a day or two, and indeed this method can be employed for nearly all the tissues and organs. It is preferable, however, in many instances to effect the process more slowly, by means of some watery fluid, such as a strong solution of bichromate of potash or a weak solution of chromic acid, since in this way the parts shrink less and consequently retain their form better; the process should, nevertheless, always be completed by means of spirit. In the case of the bloodvessels an immersion for a fortnight or three weeks in a 1 per cent. solution of bichromate of potash answers best; the pieces are then placed for a

day in weak spirit, and finally transferred to strong methylated alcohol. Here they may remain without detriment until it is convenient to prepare the sections.

The method of cutting and preparing sections for the microscope is in the main the same for nearly all the tissues and organs of the body, and since, in the further study of these, it will be in almost constant requisition, it will be convenient to give in this place an account of the mode usually adopted, reserving any special modifications which it may be needful to make in the preparation of particular organs until these come to be treated of.

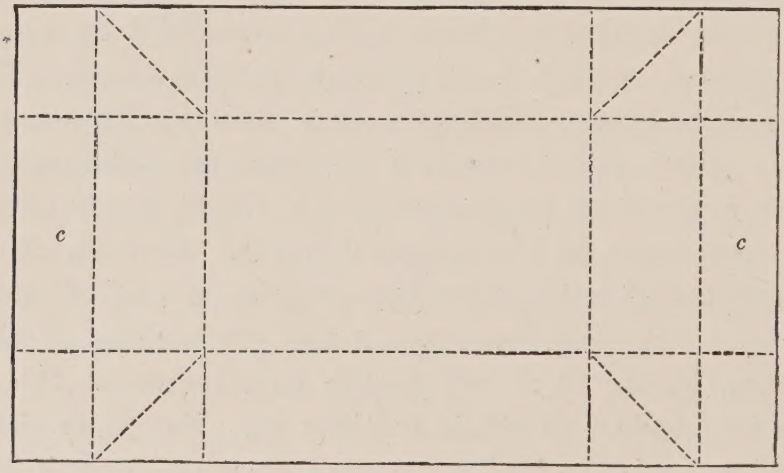
METHOD OF PREPARING SECTIONS.

Preparation of an embedding mass.—Unless the piece of tissue is large enough to admit of being held firmly by the left hand while sections are made with a razor held in the right hand, it is necessary to support it in some way. This is usually done by surrounding it in a mould with some fatty mixture which requires only a moderate temperature to melt it; the mixture or mass should, when cold, be of about the same consistence as the hardened tissue. Perhaps the most generally useful mixture for this purpose is one of wax and olive oil. This can, by varying the proportions of the ingredients, be suited at will to the consistence of the tissue which it is desired to obtain sections of. For most purposes the following is the best proportion to adopt, and it is to be observed that whenever subsequently the 'wax-mass' is mentioned, this particular mixture is indicated:—Sixty cubic centimetres of olive oil are poured into a measuring-glass, and small lumps of white wax are added to the fluid until it occupies a hundred cubic centimetres. It is now placed in a porcelain or tin vessel (a small oil-can answers the purpose very well), and heated over a water-bath until the wax is entirely melted. The mixture is then thoroughly stirred, and put aside until wanted. In laboratories where it is likely to be much in requisition it is well to have a supply always ready melted.

This may be done by keeping it in a sandbath at a temperature of about 40° C.

Process of embedding.—One or more shallow oblong moulds of different sizes should be kept in readiness for embedding pieces of tissue in. They may be made of thin pliable metal, such as sheet lead or capsule metal (fig. 23), so as to permit the sides to be readily bent back and the wax-mass removed when set; but if these are not at hand a suitable mould may always be extemporised out of ordinary stiff paper. A piece is cut of about the size and shape shown in the accompanying diagram, fig. 21, and folded along the lines there marked out, the small dotted diagonals at the corners being scored with some blunt-pointed instrument, an ordinary hard

Fig. 21.



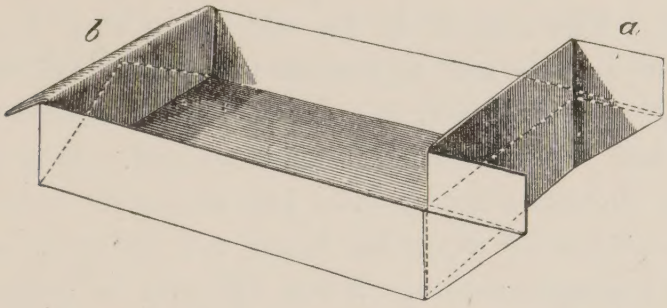
Outline shewing the manner in which a small piece of paper is to be folded to make an embedding trough.

leadpencil, for instance. These corners are then pinched up between the finger and thumb, bent round so as to be applied to the ends of the oblong (fig. 22, *a*), and are fixed there by turning down the flaps *c c* (fig. 22, *b*).

The trough being ready, it is placed upon a flat cork, and the next thing is to take the piece of tissue (artery, for example) out of spirit and place it for a minute or two on clean blotting-paper to remove the excess of fluid; at the

same time its surfaces should on no account be allowed to become actually desiccated. A minnikin pin is then passed

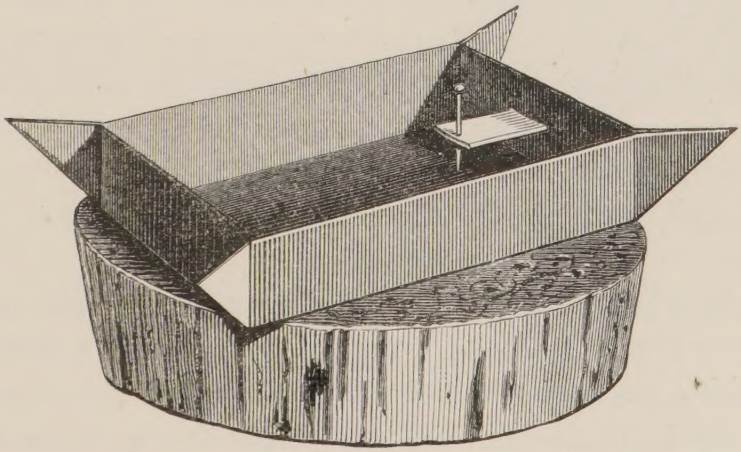
Fig. 22.



Embedding trough made from a piece of paper of the size shown in fig. 21, with one side, *b*, completed; the other, *a*, only half finished; so as to show the manner in which the corners are folded and fixed.

for a good part of its length through the piece near the end furthest from that which it is desired to cut, and the point of the pin is stuck through the bottom of the mould into the cork in such a way that the tissue is placed at one end

Fig. 23.



Embedding trough of capsule metal placed on a cork, and with a piece of tissue (artery) *in situ*. All that is further necesssary is to fill the trough with melted wax-mass.

of the oblong, and sustained in a horizontal position about half way down in the embedding trough, as shown in fig. 23,

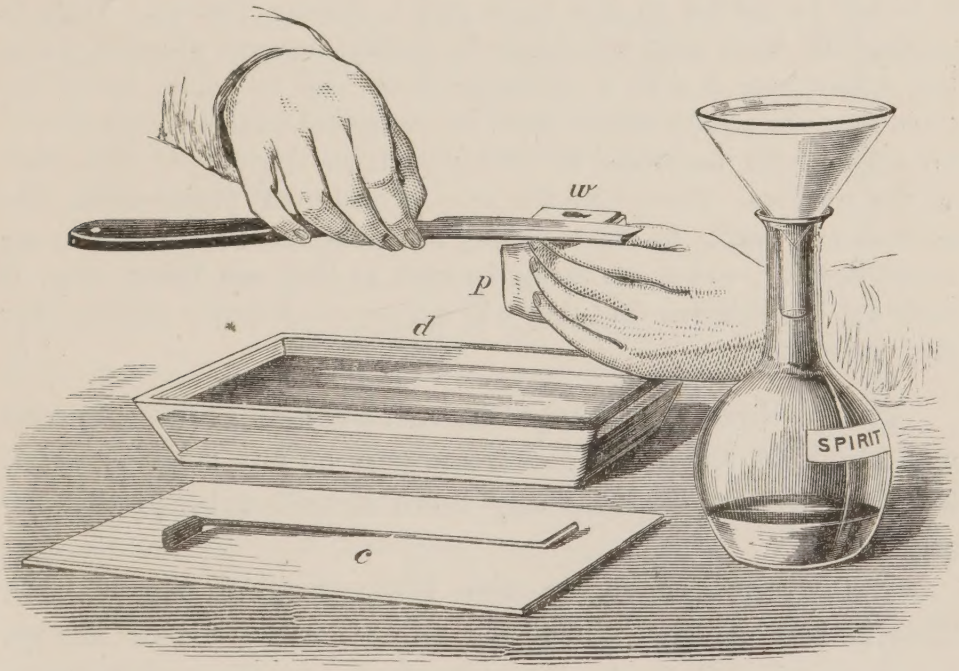
All is done as quickly as possible, so as not to allow the tissue time to become too dry (if there is any fear of this it must be moistened now and then with a drop of spirit), and without manipulating the piece more than can possibly be helped. The object to be cut being thus fixed in the proper position, melted wax-mass is poured into the mould so as to fill it completely, and entirely to surround the piece of tissue, and the mould is set aside to cool. It is important not to use the mass hotter than just above its melting-point, as the tissues, even when hardened, are liable to be injured by too high a temperature. When the wax is completely set the pin is carefully withdrawn and the trough removed from the cork and placed in spirit. Here the cooling is soon completed, and the paper or capsule metal having been taken away the embedded tissue is ready for cutting.

Method of cutting the sections.—The sections, unless a microtome or section instrument is used, when a special form of knife is often provided, are made with an ordinary razor, the blade of which should be somewhat hollow-ground on both surfaces. Some prefer that the under surface should be ground flat, and this is advantageous for large pieces of tissue, but it is not so readily kept sharp. For this last-mentioned purpose a good strop is absolutely necessary. The razor is kept constantly wetted during use with spirit, contained in a large shallow glass dish, into which the instrument is dipped from time to time, and in which also the sections are in the first instance placed.

All then being ready, the cake of wax-mass (protected from the warm fingers by being wrapped round with a small strip of bibulous paper) is held in the left hand with the end in which the tissue lies uppermost, whilst the razor is held in the way shown in fig. 24, and the wax-mass is evenly and gradually shaved off layer by layer until the embedded tissue comes into view. The pieces of wax-mass which are removed in this way must not be allowed to fall into the spirit, but are to be put on one side, and the razor

wiped clean. It is then again dipped in the spirit, and a fresh layer of the cake removed, including a moderately thin section of the tissue. This is not to be kept, the only object in making it having been to prepare a clean even surface to cut from. The razor is again wiped clean—this must be done before each fresh section—re-wetted with spirit, and, starting at the edge of the piece of tissue, a section as thin as possible

FIG. 24.



Process of cutting sections of an embedded tissue.

w, cake of wax-mass, with the piece of tissue seen in it as a dark patch, completely enclosed and supported by the wax; *p*, bibulous paper wrapped round the wax-mass, except where this is being cut; *d*, dish containing spirit; *c*, glass cover with section lifter lying upon it. The flask on the right of the figure serves to receive the spirit which has been used, for this may be employed again and again if filtered back after use.

is made, and removed on the razor blade without bringing away more of the wax-mass than can be helped. The section, if it seems thin enough, is floated off into the dish of spirit, and another and another are made in the same manner. No difficulty will be experienced in getting rid of any wax that may be still adherent to the sections; a touch with a needle is generally sufficient.

The attainment of dexterity in cutting thin sections in this manner with the free hand—that is to say, without the aid of any special instrument—is in great measure, as may well be supposed, a matter of practice. However, one or two hints drawn from experience may not be superfluous.

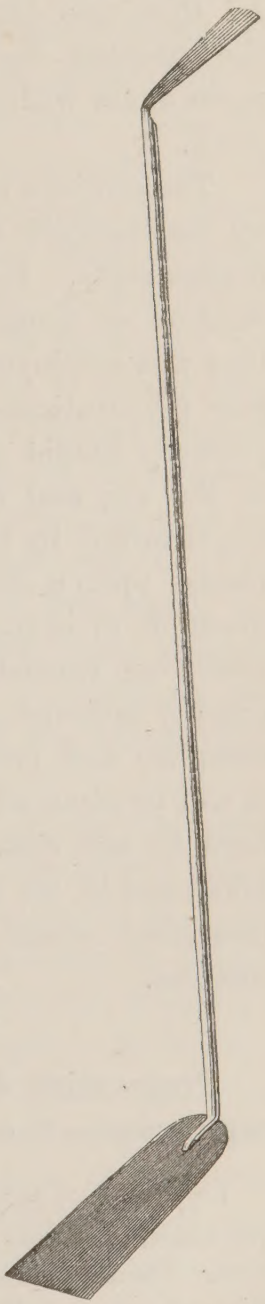
In the first place the razor must be always kept very sharp: this is the main secret of thin sections. It should also, at the moment of cutting, always have a flood of spirit upon the upper surface. The object of this is to keep the section free as it is being made, otherwise it is apt to adhere to the razor and to become broken. Then, in cutting, the razor must not simply be pushed forward over the tissue, but drawn over it with a movement from heel to point; but at the same time the whole section must be completed with one such sweep, for a to-and-fro movement inevitably produces ridges upon the surface of the section. Except in the case of injected preparations, the sections can hardly be cut too thin; as a rule, those sections only are worth keeping, which can barely be seen as they are being cut. It must not be forgotten that they will appear considerably thicker after they are stained.

Two or three sufficiently thin sections having been in this way obtained, the embedded tissue may be put aside in spirit for the present, in case any more should subsequently be required.

The sections have, in the first place, to be stained. This is best effected, in the case of the sections of artery, by immersing them in the ordinary logwood alum solution for a few minutes. They are then put into water for a second or two, to wash away the excess of the staining fluid, which would otherwise be precipitated, and are transferred from this to spirit (absolute alcohol is generally recommended, but strong methylated spirit is sufficiently anhydrous) in which they are allowed to lie until it is certain that all the water is replaced (five minutes is generally amply sufficient), after which they are placed in oil of cloves for a minute or two. In this the sections should almost immediately become clear and transparent, and the change has at the same time the effect of causing their colour to appear darker. If any parts of the section do not participate in the change of ap-

pearance, but remain opaque and cloudy, it is a sign that all the water has not been extracted, either because the sections were not left long enough in spirit, or because this was not anhydrous enough (for oil of cloves will not mix with water nor with alcohol which contains more than a trace of water). But it is not too late to put them from the oil into strong spirit again, and when they have been long enough there to transfer them once more to oil of cloves. All these transferences may be effected by aid of a section-lifter (fig. 25), made from a piece of copper or german-silver wire flattened out at one or both ends, which are bent over so as to be hoe-shaped. By means of this instrument even very thin and fragile sections may be readily lifted from one vessel to another without injury. The staining fluid, which should always be freshly filtered, and not be used for too many sections, may be in a watch-glass, which should be covered over by another watch-glass inverted, but the other fluids which are constantly in use it is better to keep in covered glass or china pots, for they—especially the oil of cloves—may be used for a considerable number of sections without detriment. The process of preparation may be arrested at any stage and completed at another time, if the sections are left in spirit or in oil of cloves.

Fig. 25.



Section-lifter. Natural size.

In transferring sections from one fluid to another, and especially when they are put in the staining fluid, care should be taken that they are actually immersed, and do not merely float on the surface.

Instead of the ordinary logwood solution Kleinenberg's fluid (see p. 176) may with advantage be used for staining sections that are to be mounted in balsam or dammar. In this case the sections can be placed from spirit into the staining fluid, and from the latter directly into the spirit again without the necessity for placing them in water at all.

The sections then being in oil of cloves, and the clearing up satisfactorily effected, all that remains is to mount them permanently. For this purpose either dammar varnish or a solution of Canada balsam in chloroform—the same liquid that was employed for cementing the coverglass of the glycerine preparations—is employed.[1] These solutions, although perfectly limpid even in the cold, readily dry when exposed to the air, and thus become hard and firm. The sections are removed by the lifter from the oil of cloves and gently placed upon a slide, the excess of oil of cloves is allowed to drain off or is soaked up with blotting-paper, a drop of the mounting varnish is placed on the coverglass, and this is then quickly inverted over the sections. The preparation is now complete and permanent, and ready for examination; but as it will be some while before the mounting varnish has become hard to any distance under the coverglass, care should be taken not to let this become shifted in any way, since such treatment would probably crumple or otherwise spoil the sections.

Preparation 4. The smaller arteries and veins and the capillaries.—The elongated epithelioid cells which form the

[1] The Canada balsam used must be heated over a sandbath until it is quite hard when cold; it is then dissolved in an equal volume of chloroform. Dammar varnish is made by dissolving one part of gum dammar in two parts of oil of turpentine, and one part of gum mastic in two parts of chloroform; mixing the solutions and filtering. The mixture is apt to become opalescent on keeping, but this disappears permanently on boiling the fluid.

walls of the capillaries, and which line the arteries and veins, are readily shown in silvered preparations of any vascular tissue. It is most convenient to choose a vascular *membrane* for the purpose, because more readily displayed, and of vascular membranes the mesentery of the frog or toad is perhaps as easy to prepare as another. The following is the mode of proceeding :—The animal (a male) having been decapitated and the spinal cord destroyed, the trunk is suspended for a few minutes by the lower limbs in order to allow the blood to drain from the body as completely as possible. The frog is then placed on its back, and the abdomen freely opened. A loop of intestine is seized with forceps and gently raised by an assistant, while with a soft camel-hair pencil, moistened with distilled water, the operator carefully brushes the mesentery on both surfaces, carrying the brush in every case *from* the intestine, not towards it. This brushing subserves two purposes—in the first place, the epithelioid cells of the serous membrane, which would obstruct the passage of the silver solution to the bloodvessels, are removed ; and in the second place, much of the blood which remains in the vessels is driven out of them.

The brushing being completed, the loop of intestine, with its included mesentery, is cut off, dipped for a second into a capsule of distilled water, and then at once placed in $\frac{1}{2}$ per cent. nitrate of silver solution. Here it is allowed to remain ten minutes ; after which it is rinsed in distilled water and then placed in common water in the sunlight. If the day is bright, the silver is soon reduced, and all that remains to be done is to place the preparation in a shallow glass dish, and carefully cut off and remove the piece of intestine, leaving the mesentery. This must be done under water, and will require sharp scissors and delicate handling, so as not to drag upon the mesentery or throw it into folds.

In order to mount it a slide is held in the water and the membrane allowed to float over, after which the slide is carefully lifted out with the membrane flat upon it, and the excess

of water is drained off. Before covering it the preparation must be examined, both with the unassisted eye and with a low power of the microscope, so as to detect any folds or creases in it. If present they can be got rid of, by gently drawing out first this corner and then that with a needle. A drop of strong glycerine may then be placed on the middle of the preparation, and the coverglass laid on and allowed slowly to settle down. More glycerine may be afterwards added at the edge if necessary. Should it be found, on examining the preparation with a moderately high power, that the outlines of the epithelioid cells of the vessels are not yet sufficiently marked, it will be well to leave the preparation out in the light, but covered over from dust, for a day or two.

If there is but little sunlight the reduction of the silver may often be better effected by placing the loop of intestine, with its attached mesentery, after it has been taken from the silver solution and rinsed in water, in a beaker of weak spirit (equal parts of water and strong spirit, freshly prepared), and exposing it to the light in this for an hour or more. The cutting off of the intestine must be performed in the same fluid and the mesentery floated from it on to the slide. The method has the advantage not only of effecting the reduction of the metal with greater surety, but also of rendering it easy to obtain the membrane free from creases, for the mesentery is partly hardened by the spirit while in a state of extension, and continues in this condition when floated on to the slide, so that it is seldom necessary further to extend it by artificial stretching. In these silvered preparations little but the epithelioid cells can be made out, for the rest of the tissue generally remains almost unstained, and becomes very transparent in glycerine.

Preparation 5.—To exhibit the muscular structure of the small arteries and veins, and the nuclei of their epithelioid lining and of the walls of the capillaries, the vessels are stained with logwood. This is done by immersing the

mesentery or other vascular membrane, either fresh, or better after having lain for a day or two in very weak bichromate of potash solution, in a dilute solution of logwood alum, until distinctly coloured; then place the tissue in water, and mount it, with the same precautions as before to prevent creasing, in glycerine. For the structure of the small arteries the pia mater from the human brain may be used. A small piece is stripped off with forceps; and as it consists almost entirely of small arteries and veins, and moreover a few capillaries are generally dragged out with it from the cerebral substance, the structure of all the vessels is, after staining, very well displayed. The small veins are here exceptional in being entirely devoid of a muscular coat, whereas the arteries have this coat well developed, and it is particularly well shown in consequence of the staining of the transverse nuclei of the muscular fibres by the logwood. Within these may be detected, by carefully using the fine adjustment of the microscope, in the first place, longitudinal striæ, which are produced by wrinklings in the elastic layer of the internal coat; and in the second place, situated most internally, elongated oval nuclei, which belong to the epithelioid cells lining the vessel. Of course, two layers of all these structures are come across in focussing from above down. The outer coat is represented merely by a few corpuscles and fibres of connective tissue which blend externally with the connective tissue framework of the membrane.

It will be found in carefully focussing from above down that at one position of the focus the small vessel has exactly an appearance as if it had been cut longitudinally through the middle, and as if the top of the lower half were being examined. The lumen is seen in the centre, with possibly a few blood corpuscles still in it; on either side of this a well-marked line representing the inner coat; outside this again what seems like a row of rounded cells, which are really the encircling fibre-cells of the muscular coat seen as if cut across; and finally, here and there, outside of all, small

cells presenting the fusiform aspect of connective tissue corpuscles seen in profile. All these appearances are exactly the same as if a section had been made along the vessel, and result from the fact that only those parts of an object which lie in the horizontal plane that happens to coincide with the focal distance of the objective, are distinctly seen, so that it seems as if only this particular slice were present. An 'optical longitudinal section' is thus obtained of the vessel.

STUDY OF THE CIRCULATION.

The study of the bloodvessels cannot be said to be in any sense of the word complete until they have been viewed in the living condition and with the blood still moving through them. Such an observation can, of course, only be made whilst an animal is still alive, and in parts which are transparent enough to allow the vessels to be distinctly seen. Membranous parts are those which are naturally best adapted for such observation, as, for example, the web of the frog's foot and of the bat's wing; the tongue, mesentery, and lungs of the frog and toad, but especially the latter animal; and the mesentery and omentum of small mammals. In such preparations the surrounding tissues, and especially the connective tissue corpuscles, may be studied as well as the bloodvessels; and the changes due to commencing inflammation which are exhibited by the bloodvessels, and the migration from the veins of the white blood corpuscles can always be brought on either by the application of irritants, or, as in the case of the serous membranes, by simple exposure to the air. The best methods, therefore, of observing the circulation of the blood in different parts will be described in the following preparations.

Preparation 6. Circulation in the frog's web.—One of the common English frogs (*Rana temporaria*), of as light a colour as possible, is chosen, and by means of a Pravaz or

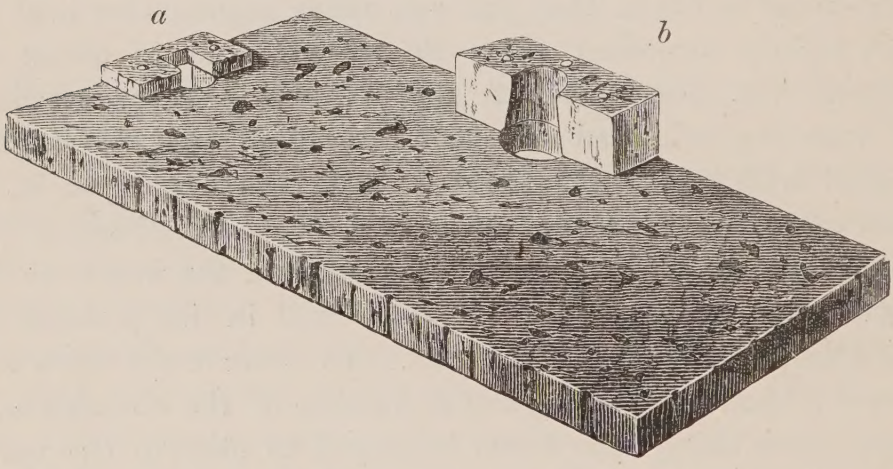
other hypodermic syringe two drops of a very weak solution of curare (1 to 2000 of water) are injected under the skin of the back. This is generally sufficient, in the course of from a quarter to half an hour, to render the animal completely motionless, whilst the pulsations of the heart and the circulation proceed unimpaired. The frog is then laid on a piece of cork or soft wood of an oblong shape which has a narrow slit at one end. One of the interdigital webs is placed over this slit and fastened on either side by means of one or two minnikin pins or fine needle-points passed through the adjoining webs. Care must be taken that the web under examination is at no part tightly stretched, since this would tend to arrest or obstruct the circulation. A slip of blotting-paper or a piece of linen rag is placed over the animal and kept thoroughly wetted with water, and the cork, with the frog upon it, is then placed on the stage of the microscope (the head of the animal being away from the observer, and the web over the aperture in the diaphragm), and is fixed in this position by the clamps like an ordinary slide. To observe the web a low power is used to see the general features of the circulation, a high power being afterwards employed to observe the parts more in detail. But this should not (unless it is an immersion) be of too short a focal distance, since otherwise the lower glass is apt to become clouded by moisture from the web. It is not advisable to put a piece of covering glass on the latter to prevent the clouding, as the circulation might thereby be interfered with or arrested.

The web of the frog's foot is the easiest of the vascular membranes to prepare, but has the disadvantage that, owing to its comparative thickness and its epidermic covering, it is not always easy clearly to make out the intermediate tissue. At the same time, being under almost completely natural conditions, the circulation will continue for an indefinite time quite unimpaired.

Preparation 7. Circulation in the mesentery.—The mesentery of the frog, and still better of the toad, is ad-

mirably adapted by its thinness and perfect transparency, as well as its great vascularity, for observations on the blood-vessels and surrounding tissues. It is necessary to have a special mesentery-plate for this purpose, which can, however, be readily made from an oblong piece of cork, like that used for the web observation. A round hole about half or three-quarters of an inch in diameter is made at one side with a cork borer, and a small piece of the same material about half an inch thick, and with a segment of a similar circle cut out

FIG. 26.



Flat piece of cork arranged as a frog-stage for viewing the circulation in the web, tongue, mesentery, or lungs.

Over the small piece of cork *a* the tongue can be fixed; *a* can be removed when the slit below it is wanted for the web; *b*, cork with a deep groove cut along one side: to this the intestine is fastened by needle-points, while the mesentery rests on a semicircular piece of glass which should fit at the top of the groove.

of its side, is fixed on the first with sealing-wax or small pins (fig. 26, *b*). A piece of glass of the same shape as this segment may be fitted into it near the top for the mesentery to rest on. The animal—a male—having been rendered insensible by destruction of the brain, or other means, is curarised as before, and laid upon its back, and a longitudinal cut about an inch long is made with scissors through the skin of the abdomen about half an inch to the right of the middle line. Before proceeding further, the operator should

wait for a minute or two to make sure that there will be no bleeding; and any blood that may have already exuded should be dried up with blotting-paper. The abdominal cavity is then opened by a corresponding cut through the muscles and peritoneum, taking care, however, to avoid any veins that may be in sight. Having again assured himself of the absence of bleeding, the operator very gently draws out one of the coils of the intestine, with its included mesentery at the aperture. The animal is now to be turned over on its side, and so propped up against the smaller cork that the wound is about on a level with the top. All that remains to be done is to place the extruded mesentery over the aperture, and to keep it in position by two or three fine needlepoints passed through the surrounding intestine into the cork. In this case again, the greatest care must be taken in no way to drag upon the exposed membrane, or to allow it to be pressed upon. Moreover, the surface must from time to time be moistened with a little salt solution, to prevent its becoming dry. But in spite of every precaution the mere exposure of the serous surface to the air is sufficient to produce before long the changes in the circulation which are characteristic of the commencement of inflammation.

Preparation 8. Capillary circulation in Mammals.—It is less easy to study the circulation in the serous membranes of mammals, for the exposure required for the purpose is apt to be far more prejudicial to the maintenance of the normal condition of the tissues than is the case with the cold-blooded vertebrates. It is necessary, moreover, to maintain the exposed part at the body temperature, and to immerse it in fluid, since it would otherwise become at that temperature rapidly desiccated. The membrane generally chosen is not the mesentery but the omentum, which in many animals, e.g. the guinea-pig, is very extensive, and at the same time thin, and provided in parts with a sufficient number of bloodvessels. The animal, which should be rather a small one, is anæsthetized with chloral hydrate, a few minims of a 50 per cent. solution being injected under the skin. The warm stage (fig. 9) is in the meanwhile got ready, and a glass tray (which can be extemporised out of a small plate of glass, some pieces

of glass rod and sealing-wax) is placed on it and filled with salt solution, which is maintained at about 38° C. Then the animal is supported on a block at a convenient level, and the abdomen having been carefully opened, a little of the omentum is drawn out and allowed to float flat in the warm salt solution, where it can be examined either with a low power or with an immersion objective dipping into the solution. If the latter be employed a piece of thin covering glass must be placed over the part of the membrane which is to be examined, so as to sink it in the fluid and keep it steady. But in spite of every precaution, the circulation under these conditions retains its normal character but a short while, and inflammatory congestion and *stasis*, or complete stoppage of the flow of blood, rapidly supervenes.[1]

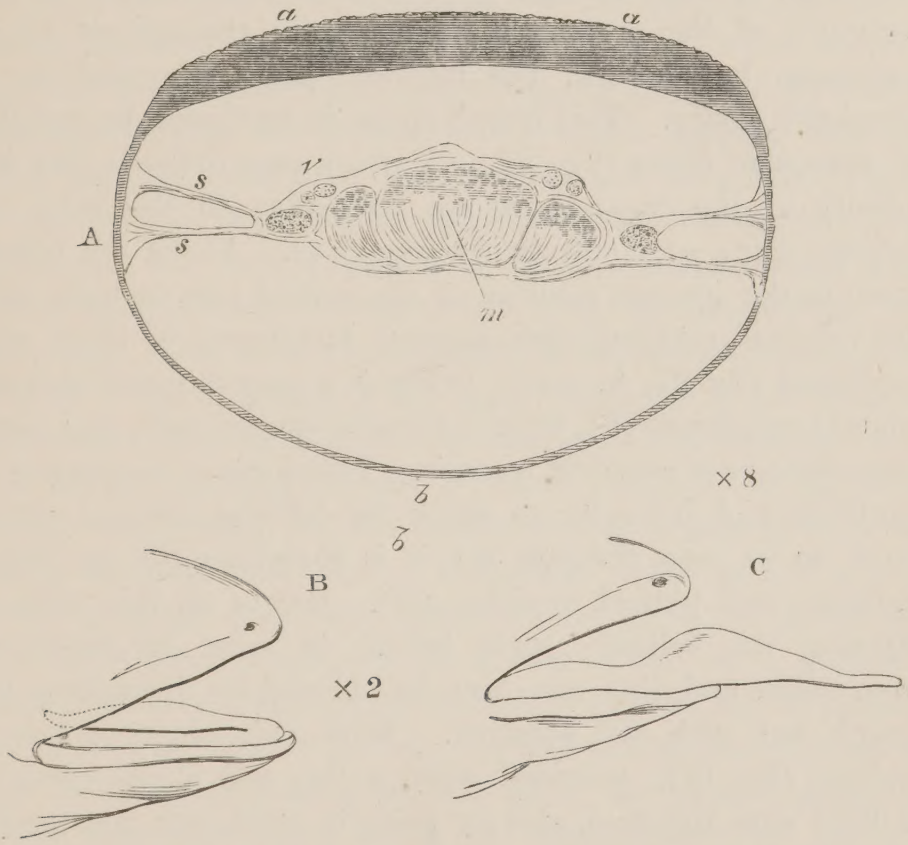
Preparation 9. Circulation in the lung of the toad.—This is readily observed with the aid of the mesentery board. The animal must, as before, be first rendered insensible and curarised, but it will be found that a good-sized toad will require at least six times as much curare as a frog. An opening is made at the side of the chest large enough to allow the lung, which in the toad almost always remains distended with air, to protrude. The animal is then propped up on the mesentery board (Fig. 26) in such a manner that the lung rests over the aperture *b*, and the circulation can be studied in the part which is uppermost without further trouble. A frog may be used in a similar way, but there is much greater difficulty in keeping the lung distended. In either case the greatest care must be taken to avoid pricking, or in any way rupturing the wall of the lung.

Preparation 10. Circulation in the tongue of the toad.—By far the most beautiful object for studying not only the circulation but also the tissues in the living animal, is the tongue of the toad, and in a slightly less degree that of the frog. The tongue is in these creatures an extremely extensile organ, which, under ordinary circumstances, lies

[1] For a detailed account of this method the student is referred to the original description by Burdon Sanderson and Stricker in the 'Quarterly Microscopical Journal' for 1870.

folded back on the floor of the mouth (fig. 27, B), but which can at the will of the animal be protruded for a considerable distance (C). For the preparation of the organ the cork plate is again necessary; a smaller piece of cork of the shape shown in the figure (fig. 26, *a*) and about one-eighth of an

FIG. 27.



Structure and position of the tongue of the toad (Dowdeswell).

A. Transverse section through the middle of the organ with the lymph-spaces fully distended; *a a*, thick, papillated mucous membrane; *b*, thin lower membrane; *m*, muscular bundle cut across, united to the sides of the tongue by septa of connective tissue *s s*; *v*, position of the larger bloodvessels.

B. Profile view showing the tongue in its ordinary position within the mouth.

C. The same when extended.

inch thick, being fastened with pins over the slit which served for the display of the frog's-web.

The toad having been rendered insensible and curarised as before is laid upon its back with its nose close to the slit.

The lower jaw is then raised and the folded-back end of the tongue is found, and drawn gently out of the mouth with forceps. The end has a pointed projection or cornu on each side; these are successively laid hold of by the forceps, and fastened with needle-points to the small piece of cork on either side of the slit. Before the rest of the operation is described, a word or two may be said with regard to the structure of the organ. It is not solid throughout as in mammals, but hollow, the interior being occupied by a lymphatic cavity. This lymph space is traversed by bundles of muscular fibres (fig. 27, *m*) which pass towards the extremity of the organ and are connected to the sides by delicate septa of connective tissue (*s s*). Above the lymph space in the present position of the animal—on its back with the tongue extruded—the mucous membrane is thick and papillated (fig. 27, A, *a a*). Below is a very thin and delicate mucous membrane (*b*), with numerous bloodvessels, and small muscular fibres running over it. The former membrane is too thick and irregular to allow the delicate internal structures to be seen through it; it is therefore slit up longitudinally and pinned to either side. But to do this without injuring the delicate parts below, it must be separated from them, and this can best be effected by distending the lymph sac with salt solution. With this object a Pravaz syringe (fig. 17), provided with a fine and sharp cannula, is filled with the fluid, and its point is stuck into the tongue near the end, passing about half an inch backwards. It will almost certainly be found that on pressing the piston down the salt solution will readily flow into the lymph sac, and as it fills this will cause the thin mucous membrane at the lower part to become bagged out, and completely separated from the muscular bundles, *m m*, and these again from the thick layer above. The latter is now carefully slit up along its middle by sharp fine scissors, and first one edge of the wound and then the other is drawn to the side of the slit in the cork and fastened there by two or three needle-points. If everything

is carefully done there will be no escape of blood over the preparation; but should any blood have exuded it may be washed off by pouring a little salt solution over the surface.

There is now brought to view the fan-like group of muscles which pass through the middle of the lymph sac, and the bundles of which are, as before mentioned, connected with the sides and with one another by delicate septa of connective tissue, traversed by a few bloodvessels; and it is this delicate connective tissue, of which two strata can generally be traced, one superficial to the other, which is better adapted than any other part for exhibiting both vessels and connective tissue corpuscles (the latter rather peculiar in form and appearance). Moreover, the mere exposure of the lymphatic surface soon causes inflammatory changes, and after the preparation has been made a few minutes only, the first commencement of these is seen in the sticking of the pale corpuscles to the walls of the vessels, speedily followed by their migration from the veins into the surrounding tissue. Nowhere can the fact be more clearly established, and the details of the process more accurately followed, than here. Moreover, if the animal is kept properly moist and from time to time given an additional dose of curare, it remains for a long while in a perfectly natural condition, and the inflammatory process can be studied for as long as may be desired. By examining such a preparation at intervals during several days, the same connective tissue corpuscles being again and again found and brought under observation, it has been conclusively proved that, at all events as regards these corpuscles of the tongue of the full-grown toad, the connective tissue corpuscles take no active part in the process of acute inflammation. The circulation of the blood among the muscular fibres can also be well seen in this part of the tongue.

Lastly, by focussing through the connective tissue septa, or by severing the longitudinal muscular bundles which they serve to unite, the vessels of the lower mucous membrane are brought into view, especially if a slip of glass is fitted into the

small piece of cork, so as to support the tongue and prevent the thin membrane from bulging downwards.

METHOD OF INJECTING THE BLOODVESSELS.

Before leaving the subject of the bloodvessels the best mode of filling them with transparent material may be described, especially as in the study of the several organs it is necessary, in order that the course and arrangement of the vessels may be properly made out, that sections of injected as well as of uninjected preparations should be looked at. It will be convenient in this place to describe the injection of a small animal entire from the aorta, reserving any special directions concerning organs which are not thereby properly injected, such, for instance, as the lungs and liver, until they are severally dealt with.

Preparation of the injection mass.—This is almost always a solution of gelatine coloured, either red with finely precipitated carmine, or blue with soluble Prussian blue. Sometimes, but rarely, when it is wished to inject two sets of vessels of different colours, both of these are used, but as a rule all the bloodvessels—arteries, capillaries, and veins—should be filled with the same injecting fluid; preparations in which the arteries are filled with one colour and the veins with another are pretty to look at, but are difficult to prepare, and present no practical advantage. The gelatine solution is made as follows:—Ten grammes of clear gelatine are cut into small pieces, and placed in a beaker of cold distilled water to soak. In about an hour the gelatine will have swollen to several times its original volume. The excess of water is now poured off, a glass cover put over the beaker, and it is placed in a water-bath and heated until the gelatine is rendered fluid.

For the *red* injection 4 grammes of carmine are rubbed up in a mortar with 8 cubic centimetres of liquor ammoniæ, and then 50 cubic centimetres of water are added. When the carmine is as completely as possible dissolved, the liquid is filtered. The process of filtration occupies some

time—several hours, in fact—and may be conveniently left over-night. The filtrate is warmed, and the gelatine solution is gradually added to it with constant stirring. The next part of the process is to precipitate the carmine, for otherwise it would diffuse through the walls of the vessels and colour the tissues; but it must be precipitated so finely that the particles shall not be visible even under the highest power of the microscope. To effect this object a small quantity of a 10 per cent. solution of acetic acid is placed in a burette and allowed to run drop by drop into the warm, carminised, gelatine solution, which is all the while constantly agitated. As its alkalinity becomes neutralised the ammoniacal odour becomes less and less strong, and eventually disappears, and is replaced by the vinegar-like smell of acetic acid. The alteration in reaction may be shown, in spite of the red colour of the solution, by placing a small drop on a piece of blue litmus paper; if the *opposite* side of the paper be looked at, it will be found to have assumed the characteristic bright red colour, which acids produce, and which is quite different from carmine. This change is owing to the diffusion of the acetic acid through the paper, whereas the carminised gelatine sets almost immediately and is thus unable to soak through.

But it is not sufficient in order to effect the precipitation of the carmine that the fluid should only just be acidulated; there must be an excess of acid. A few more drops are therefore added, and the carmine thrown out of solution. This change from the soluble to the insoluble state is accompanied by a very marked alteration in colour, for whereas whilst still in solution the carmine imparted the rich deep red of an ammoniacal solution to the gelatine, after the precipitation the colour of the latter changes to a paler red, comparable rather to the tint presented by the powdered carmine in the dry state. Even after the production of this change a few more drops of the acetic acid may be added, for it will do no harm, and will tend to counteract the natural alkalinity of the tissues.

The coloured gelatine is next filtered through a piece of flannel or fine linen, previously soaked in hot water, and again wrung out, and is collected in a flask as it runs through the filter, and transferred to the injecting bottle.

For the *blue* injection 10 grammes of gelatine are taken, and after having been soaked in cold water and dissolved up as before, 50 c.c. of a 2 per cent. solution of Berlin blue, which has been previously warmed, is gradually added with constant agitation to the fluid gelatine. The blue mixture is filtered and is then ready for use, without the necessity of precipitating the colouring matter, for this being a colloid is indiffusible.

It is sometimes advantageous in cases where the structure of the walls of the bloodvessels is to be the subject of observation, to use an injecting mass which is far less deeply coloured. This can of course be readily obtained by diminishing the proportion of carmine or Berlin blue which is used.

The soluble Berlin blue is of great value for the purpose of injecting both the bloodvessels and lymphatics. Unfortunately it is very troublesome to prepare. The following is the method recommended by Brücke, to whom we owe its introduction :—

Take of potassic ferrocyanide 217 grammes, and dissolve in a litre of water (solution A).

Take a litre of a 10 per cent. solution of ferric chloride (solution B).

Take four litres of a saturated solution of sulphate of soda (solution C).

Add A and B each to two litres of C. Then with constant stirring pour the ferric chloride mixture into the ferrocyanide. Collect the precipitate upon a flannel strainer, returning any blue fluid which at first escapes through the pores of the flannel ; allow the solutions to drain off ; pour a little distilled water very carefully over the blue mass, returning the first washings if coloured, and renew the water from day to day until it drips through permanently of a deep blue colour.

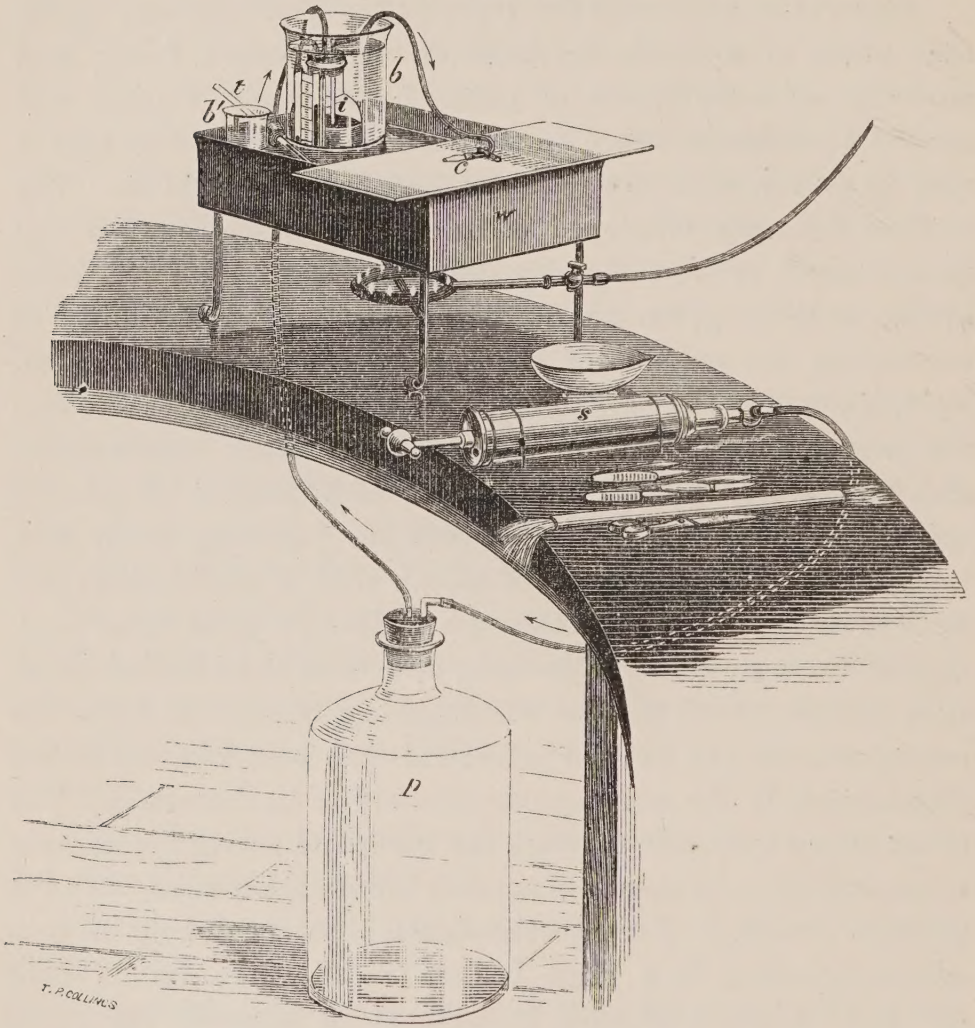
This is a sign that the salts are washed away, and all that is further necessary is to collect the pasty mass from the strainer and allow it to dry.

Apparatus employed for injecting.—This consists, in the first place, of a bottle for holding the coloured fluid; and secondly, of some means of producing a steady, elastic, and readily alterable pressure on the surface of the fluid so that it may be driven with any required force into the arteries. The method formerly employed of forcing the injecting material from a small syringe directly into the bloodvessels has been almost entirely given up, on account of the impossibility of estimating the amount of pressure which is being exerted, leading often to the employment of too great a pressure and the consequent rupture of some of the smaller bloodvessels. Fig. 28 represents a convenient form of apparatus for general use. The bottle (*i*),[1] which holds the injecting fluid, is a moderate-sized, wide-mouthed phial, with a well-fitting vulcanised india-rubber cork, through which two glass tubes pass. One of these goes to the bottom, and from it an india-rubber tube passes, which will be subsequently connected with the artery cannula (*c*), but not before this has been inserted in the bloodvessel, in the manner immediately to be described. The other passes only just through the cork, and serves to maintain communication by means of another india-rubber tube, with the pressure-bottle *p*. The injection-bottle is placed during the process of injecting in a large beaker (*b*) of warm water (about 40° C.); a piece of cork is wedged in between the bottle and the side of the beaker to prevent the bottle from floating up as it becomes emptied of injection, and the beaker is covered with a glass plate (not shown in the figure). The pressure-bottle is a large glass or earthenware bottle capable of holding two or three gallons, and tightly fitted with an india-rubber cork, through which two glass tubes pass. One of these is connected, as before mentioned, with the injection

[1] A similar one is better shown in fig. 30, at c.

bottle, and the other with a condensing syringe (*s*), by means of which the air within the bottle can be brought to any

FIG. 28.



Injecting apparatus. Complete.

s, condensing syringe, fixed to the table; *p*, pressure bottle; *b*, beaker of warm water in which the injection-bottle, *i*, stands; *b'*, small beaker containing salt solution; *w*, water-bath heated by a ring burner below: the temperature of the water is indicated by a thermometer *t*, placed in it; *c*, arterial cannula, connected to an india-rubber tube from the injecting-bottle: close to the cannula is a steel clip. The cannula rests upon a glass plate, which may serve either to put the animal which is being injected upon, or to cover it over, if it is thought necessary to place it in the waterbath.

state of tension that may be desired. Finally, if the injection is to occupy a considerable time, a water-bath or sand-bath

(*w*), heated by a ring-burner to about 40° C., should be provided for receiving both the beaker containing the injection-bottle and the animal, and maintaining their temperature during the process. Ordinarily, however, if the operation be quickly and dexterously performed, the whole process will not occupy more than a few minutes, and will be over before the natural heat of the body has had time to become dissipated.

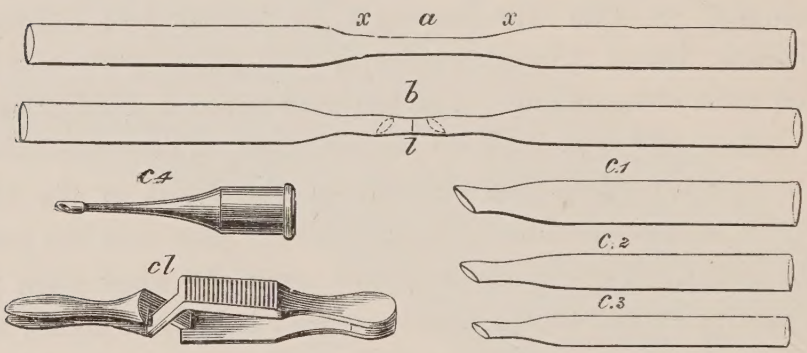
Everything then being in readiness, the animal, a rabbit, guinea-pig, or rat, for example, is killed by chloroform inhalation, being placed under a bell-glass with a sponge wetted with chloroform. The moment it has ceased to breathe it is taken out and held by an assistant, whilst the operator first quickly reflects the skin from the front of the thorax and then makes an opening in that cavity just over the position of the heart. This is then seized near the apex with blunt forceps, drawn out of the aperture, and held here by an assistant. The aorta is then found, the point of a pair of forceps passed under it close to the heart, and a thread ligature drawn round it. A snip is now made in the left ventricle, and an arterial cannula (fig. 29, *c'*) passed through this into the aorta, in which it is tied by the ligature. Then by means of a pipette a little warm water or salt solution is passed into the cannula so as completely to fill it to the exclusion of air.

The next thing to do is to connect the cannula with the india-rubber tube which brings the injection from the bottle. But this tube must first be completely filled by the injection, so that it contains no bubble of air. To effect this, whilst the india-rubber tube is kept closed by the strong spring clip with which it is provided, the air in the apparatus is put under a pressure of about two inches of mercury by working the syringe. The free end of the india-rubber tube is now held up, and the clip opened until the coloured fluid forced up by the pressure begins to escape, when the clip is immediately closed, and the tube is slipped on to the arterial cannula. The greatest care must be taken throughout to avoid the

introduction of air, since this would obstruct the smaller vessels and prove fatal to the success of the injection.

The clip is now permanently opened and the injection suffered to flow into the aorta, at first under the low pressure of two inches of mercury; but the pressure is gradually increased by working the syringe until a pressure of four or five inches is attained. The blood in the vessels gets

FIG. 29.



Cannulas for injecting. Natural size.

$c.^1$, $c.^2$, $c.^3$, glass cannulas of different sizes; $c.^4$, metal cannula: it is sometimes more easy to insert than the glass ones, especially into fine bloodvessels, or into lymphatics; *cl*, steel clip for clamping an artery, or a small india-rubber tube; *a* and *b* are intended to illustrate the mode of making the glass cannulas; *a*, glass tube which has been heated in the middle in the blow-pipe flame, and drawn out so as to be narrower here; *b*, the same tube after having been again heated (by the tip of the flame), and drawn out at the points *x x*, so as to narrow it still more at those places. The subsequent proceeding consists in making a nick at *l* with the edge of a file, breaking the tube across here, and with a fine, flat, wetted file grinding the ends away obliquely as far as the dotted ring in each. The sharpness of the filed edge is got rid of by inserting it for a moment or two in the flame. Two similar cannulas are thus made from the one piece of tubing.

forced before the injection into the right cavities of the heart, so that these are soon much distended; when this is the case the right ventricle is slit open and the accumulated blood allowed to flow out. The blood is soon followed at first by a mixture of blood and the coloured gelatine, but afterwards by the latter only; after this has been escaping for a minute or two the slit in the ventricle is closed by placing a clip on, or tying a tape round the heart, and the

injection, being thus obstructed in its outflow, accumulates in the vascular system, and distends all the vessels to their fullest extent. The success of the injection may be estimated by the colour of those parts which are not concealed by the fur; the paws, lips, nose, and ears, for instance, and the tongue and interior of the mouth. After waiting a few minutes longer, until the vessels may be considered to be completely filled, a tape ligature is put round the base of the heart, so as to include all the great bloodvessels, and is slowly tightened. This will effectually prevent any escape of the fluid gelatine from the vessels when the cannula is removed from the aorta, which may therefore now be done, the clip having been first replaced on the india-rubber tube which is connected with it, so as to prevent the injection from spirting out.

The animal is now put aside until it has become quite cold and the gelatine is firmly set. The abdomen is then freely opened, and the skull-cap removed (if the brain is wanted), and incisions having been made through the skin of the limbs here and there, the animal is placed overnight in weak spirit (equal parts of water and spirit), and the next morning those parts and organs which their colour shows to be well injected are carefully dissected out and placed in rather stronger spirit. In another day or two they may be transferred to the strongest spirit, in which they will soon become hard enough for the preparation of sections. The object of thus gradually increasing the strength of the spirit is to prevent as much as possible the shrinking of the gelatine which otherwise results.

The blue injection possesses the material disadvantage that it is apt to become temporarily reduced in the smaller vessels and rendered almost entirely colourless, so that it is difficult to determine whether a successful injection has been made or not. The colour may, however, be readily restored by pouring some oxidising fluid, such as a weak solution of peroxide of hydrogen, over any part about which there is

doubt; and in the ordinary course of preparing and mounting sections, the blue colour is always brought back, especially if turpentine is substituted for oil of cloves.

Most other forms of apparatus which are used for injecting are more or less like the one above described, the chief modification being in the mode in which the pressure is produced, this being effected in one form by allowing water to flow from a tap into the pressure-bottle (which in such cases is generally made of metal), and thus compressing the air; in another by allowing mercury to flow from one vessel into another. But the latter method, although useful when small quantities of injecting fluid only are required, as with the injection of the lymphatics (with which the apparatus will be described), is costly for large quantities; indeed it will be found that none are more simple and efficient in working than the one here recommended.

If a condensing syringe is not at hand, sufficient pressure may be got in many cases merely by *blowing* air into the pressure-bottle through an india-rubber tube, its escape being prevented by subsequently clipping the tube.

Injections which are fluid in the cold (of which the best is a one or two per cent. solution of Berlin blue) are sometimes used for the bloodvessels, especially for injecting cold-blooded animals, but they do not as a rule yield such good results as a successful gelatine injection.

Any of the coloured gelatine that may remain over can be preserved (even for a considerable time) until again wanted, if the precaution is taken, after disconnecting from the pressure-bottle and allowing the fluid in the cannula tube to run back, to place the bottle, tubes and all, for a few minutes in boiling water, and whilst still hot to stopper up the ends of the tubes with pieces of glass rod. The whole can then be put away until wanted; but it is as well to heat it up now and again in boiling water, to destroy any germs of fungi which may perchance have entered the bottle.

CHAPTER IX.

LYMPHATICS AND SEROUS MEMBRANES.

It is in preparations of the serous membranes that the structure and arrangement of the lymphatic vessels can be best demonstrated, and it will on this account be convenient to combine them here under one head, especially as the method which on the whole exhibits the structure of the serous membranes best is the only one which shows at all satisfactorily the structure of the lymphatic vessels and their relation to the cell-spaces of the connective tissue.

Preparations 1, 2, and 3. Preparation of the rabbit's omentum.—A rabbit having been killed by bleeding, the abdomen is opened, and the omentum, which is generally to be found crumpled up close beneath or to the left of the stomach, is raised with forceps, cut off as close to the line of attachment to the stomach as possible, and placed in a shallow dish of salt solution which is at hand to receive it. Besides this salt solution there should be ready on the table a little ½ per cent. solution of bichromate of potash in a bottle, some ½ per cent. solution of nitrate of silver, a wash-bottle of distilled water, a flat glass dish containing a mixture of spirit and water (equal parts), two glass plates about four inches by six, a large soft camel-hair brush, and two or three clean capsules and watchglasses.

Two small corners are first to be cut off the omentum. One of these is placed in the bichromate of potash, put aside, and examined after two or three days; this is for

exhibiting the arrangement of the connective tissue fibres. The other is first rinsed with distilled water; then placed for one minute in a watchglass containing a little of the silver solution; rinsed again with distilled water, and exposed to the sunlight in another watchglass containing water. After from a few minutes to half an hour of exposure, according to the intensity of the light, it may be removed, and a portion or the whole of it cautiously mounted by being floated upon a slide under water. The excess of water is removed from the slide, all creases and folds are carefully got rid of in the same way as with the frog's mesentery before described, and finally a drop of glycerine is added and the coverglass superposed. This preparation is for the purpose of showing the epithelioid layer which covers each surface of the membrane. If only a portion were mounted, the rest may be placed for a few minutes in weak logwood before mounting; in this way the nuclei of the cells may be brought to view.

But while this second corner was being exposed to the light the preparation of the rest of the omentum can be proceeded with.

In the first place, it is floated on to one of the glass plates and removed from the fluid, and then by drawing gently first at one place and then at another the creases and folds are gradually removed, and it is in this way spread out as an exquisitely delicate membrane which may be made to cover the whole upper surface of the glass plate, and may be extended round its edges so as to reach the lower surface. As soon as all folds are in this way removed from the part which covers the upper surface of the plate the second glass plate is applied to the under surface of the first, and the membrane, or at any rate its greater part, is thus maintained in an extended state. Next, the surface is gently brushed all over with the camel-hair pencil moistened with salt solution; this is for the purpose of removing the epithelioid layer from that surface, and enabling the silver solution more rapidly to penetrate. The brushing is not absolutely essential, for in

many parts, especially those in which the lymphatics and bloodvessels are most numerous, the epithelioid layer is deficient, or at least incomplete and modified.

The salt solution is now thoroughly washed from the membrane with distilled water, and without delay a quantity of nitrate of silver solution is poured on it and allowed to run over every part of the exposed surface. After five minutes it is again washed with distilled water, and the glass plates, with the membrane of course still upon the surface of the upper one, are placed in the weak spirit contained in the flat glass dish. The dish is then covered and placed in the sunlight until the silver, as evidenced by the change in colour, is fully reduced. Small pieces, each about an inch square, are then cut with sharp scissors from various parts, floated on to slides with the browned surface uppermost, and are exposed to the air for a few minutes to allow most of the *spirit* to evaporate, leaving them in water; to this a drop of glycerine is added, and finally the coverglass is superposed. Or the coverglass may be put on first, and then glycerine placed at the edge and allowed to diffuse in underneath the cover. The preparation is completed by cementing the covering-glass in the usual way.

Both before and after the treatment with nitrate of silver it may have been noticed that the delicate membrane is studded all over with patches of thicker tissue, some quite small and insular, others extending over a considerable area. These patches, which are characterised by an accumulation of lymphoid cells and by the small size of the epithelioid cells of the surface (there being, in fact, in many cases, a loss of distinction between them and the other cellular elements of the membrane), are, at least the larger ones, provided with numerous bloodvessels, the epithelioid lining of which is often very well stained by the silver; and these are always accompanied by one or more lymphatic vessels, with walls formed by the characteristic wavy outlined cells.

Preparations 4 and 5. The central tendon of the dia-

phragm with its serous coverings.—The thoracic and abdominal surfaces of the diaphragm present important differences in the arrangement of the numerous lymphatic vessels which are distributed upon them. To see both properly it will be necessary to sacrifice two animals. It is best to use rabbits, since their central tendon is larger in proportion than that of most other mammals.

For the preparation of either side both thoracic and abdominal cavities must be freely opened, the ventral attachment of the diaphragm being left intact, so that that muscle remains stretched out. A double ligature is to be put on the inferior vena cava in the thorax, and the vessel cut between the two threads; this is to prevent the blood in the vessel from getting over the membrane. The pericardium is now cut away from the upper surface of the diaphragm, and the suspensory ligament of the liver from the lower. The surface which it is wished to silver is then brushed pretty firmly with a camel-hair pencil wetted with distilled water, after which a few drops of nitrate of silver solution are allowed to flow over it, or are applied with the brush. After five minutes' contact the silver solution is washed off by a stream of distilled water, and the central tendon, including also some of the muscular fibres which converge to it, is carefully removed, pinned out upon a loaded cork or cake of wax, with the silvered surface uppermost, and exposed to the sunlight either in water or weak spirit. When distinctly browned it is removed from the window, and pieces from different parts are cut out and mounted in glycerine.

In addition to these preparations—which exhibit more especially the lymphatics and cell-spaces of the serous membrane, and, on the abdominal side, the lymphatic clefts between the tendon bundles—it is useful to make another silvered preparation, unbrushed, of the peritoneal surface, a third animal being sacrificed for the purpose. This serves to show the epithelioid layer of the serous membrane, with the differences in character of its cells in different parts, the cells

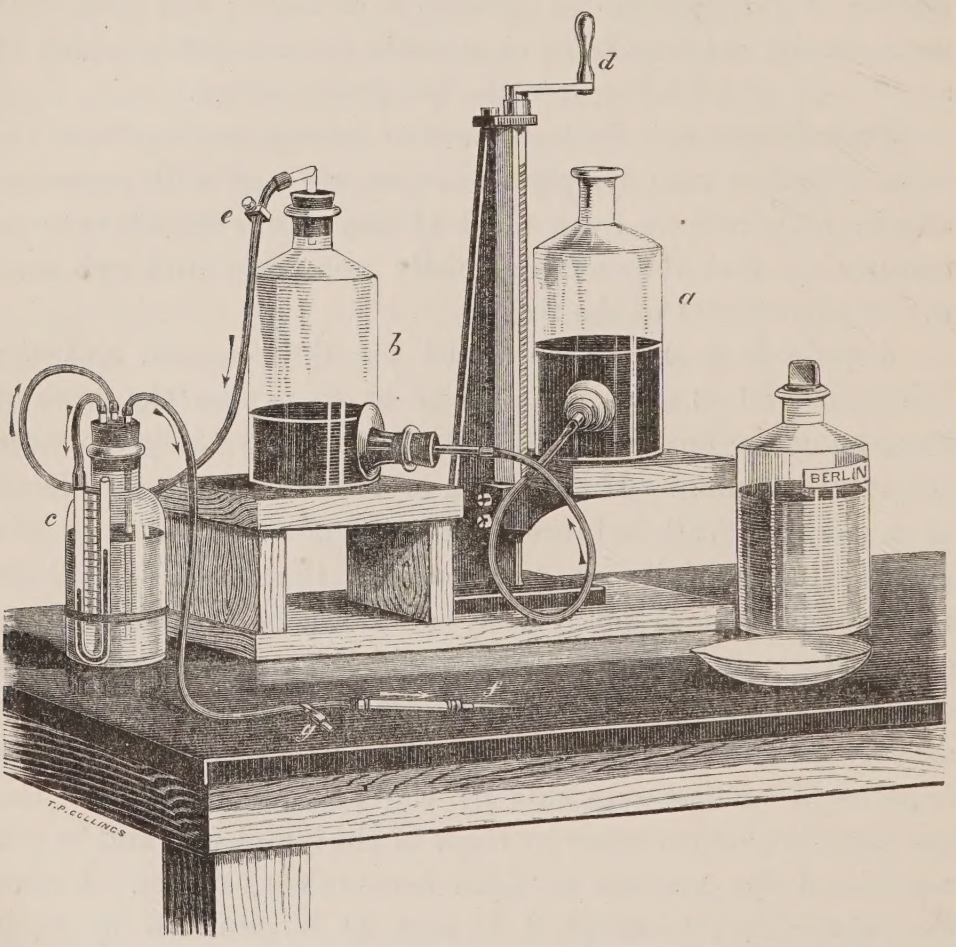
being much smaller over the interfascicular lymphatics than elsewhere. Amongst the smaller cells, moreover, may be seen here and there the minute darkly-stained angular patches known as pseudostomata, which are probably merely accumulations of intercellular or ground substance; and also, but more rarely, the true holes or stomata surrounded by a ring of small cells, and leading into the lymphatic below.

Preparation 6.—Such stomata or orifices leading from the serous cavities into lymphatic vessels are met with occasionally in preparations from most of the serous membranes in mammals. But they are especially numerous and well seen in the peritoneum of the frog.

A male frog should be killed for the purpose, and the intestines and stomach removed so as to expose the back of the abdomen, but without cutting the mesentery too near the spinal column. If the trunk of the animal is now placed in a dish of salt solution, and the posterior part of the peritoneum carefully examined under that fluid, it will be found that it does not closely cover the vertebral column, great vessels, and other structures which are found at the back of the abdomen, but is separated from them by a large lymph-space, divided from the serous cavity by a membrane. This is covered on the one side by the epithelioid cells of the peritoneum; on the other by those of the lymphatic, and to the unassisted eye appears to form a complete septum. Under the microscope, however, it is seen to be studded by very numerous apertures, which can be seen if the membrane is removed and examined with a high power in salt solution merely. But to study their arrangement with reference to the epithelioid cells, and also to obtain a permanent preparation, the septum is to be stained with silver. With this object the whole, or a portion only, is dissected off under salt solution (it may be convenient to remove with it the elongated kidneys which adhere to it behind and to cut these away only after the staining is completed); it is then rinsed in distilled water to remove the salt; placed in silver solu-

tion for one minute; again rinsed, and exposed in water to the light. After the metal is reduced the preparation is floated on to a slide, and, with the usual precautions to

FIG. 30.



Mercurial-pressure apparatus for injecting lymphatics.

a, reservoir-bottle containing mercury; *b*, pressure-bottle into which this tends to flow; *c*, injection-bottle containing solution of Berlin blue, connected with the pressure-bottle by one india-rubber tube, with the cannula *f* by a second, and with a small manometer by a third; *d*, handle of screw, by turning which the stage on which the bottle *a* rests is raised or depressed, and the pressure increased or diminished in *b*; *e*, screw clip (opened); *g*, spring clip (closed).

avoid folds and creases, finally mounted in glycerine. It is desirable, before mounting, to stain the tissue with log-wood in the way recommended for the unbrushed silvered piece of the rabbit's omentum, but this is not absolutely

necessary, and much increases the risk of producing folds in the membrane.

For studying the structure of the larger lymphatic vessels, as, for example, the thoracic duct, precisely the same methods, both for teased preparations and for sections, are employed as were used for the larger bloodvessels.

INJECTION OF LYMPHATICS.

The minute lymphatics of a part may, where numerous, generally be readily displayed by simply sticking a very fine cannula into the tissue, and forcing a coloured fluid through this. The best apparatus for the purpose of obtaining the requisite pressure is the small mercury apparatus shown in the accompanying figure (fig. 30). The mercury contained in the bottle *a* compresses the air in the pressure-bottle *b*,

FIG. 31.

Very fine perforated steel needle for injecting the lymphatics of a part.

according to the height *a* is raised above *b*, this height being regulated with the greatest nicety by the screw *d*. The bottle *c* containing the injection fluid communicates by one tube with the pressure-bottle, and by another (which passes to the bottom) with the injecting cannula *f*. Gelatine is not used for injecting the lymphatics, but almost always injections which are fluid in the cold. Berlin blue solution (2 per cent.) is often employed, but the best fluid for the purpose is a solution of alkanet in turpentine, which readily flows into the lymphatics. The cannula can be made from a piece of glass tube drawn out to a capillary point, but the best are long perforated steel needles like those supplied with the Pravaz syringes, and as fine as it is possible to procure them (fig. 31). The india-rubber tube connected with the cannula is closed by the clip *g*.

Preparation 7.—The mode of injecting the lymphatics of a tendon may be here described as an example, especially as the subject was omitted when studying the minute structure of tendon. One of the best tendinous structures to choose for the purpose is the fibrous aponeurosis covering the tendon of the triceps extensor femoris of the dog. Two sets of lymphatics are here met with—one in the substance of the tendon, consisting for the most part of vessels arranged conformedly with the direction of the fibres and connected at intervals by transverse branches, so as to form elongated and oblong meshes; and a superficial one in the areolar sheath which covers the aponeurosis, consisting of vessels forming a close plexus with polygonal meshes. The latter plexus should first be attempted. Both tube and cannula being completely filled with the injecting fluid to the exclusion of air-bubbles, the clip *g* is closed, and the cannula is inserted obliquely for half an inch or more into the areolar sheath, care being taken not to mess the surface of the tissue. This will probably be effected after one or two trials, provided the cannula is sufficiently fine and sharp. The clip is then removed, and by turning the handle *d*, and thus raising the bottle *a*, the pressure is put on to about an inch of mercury, as indicated by the gauge attached to the injection-bottle. If the insertion of the cannula have been fortunate, the blue or red fluid will almost immediately begin to pass into the lymphatic plexus, but should there be no result the pressure may be gradually raised to about two inches; higher than this it is not as a rule advantageous to go. If there is still no result the cannula may be pushed a little further in the sheath, and perhaps moved a little to one side or the other in the hope of thus rupturing a lymphatic and gaining an entrance into the plexus. Should these and other devices which experience may suggest still fail, the clip must be replaced and another insertion tried elsewhere. It very frequently happens that the injection which escapes from the end of the cannula, instead of passing into the lymphatics,

forms merely a bulla of extravasated fluid in the interstices of the tissue. This can sometimes, by passing the handle of a scalpel over it with moderately firm pressure, be induced to find its way into the absorbent vessels, but if not the cannula must be withdrawn and re-inserted as before.

For the lymphatics in the fibrous substance of the aponeurosis the cannula must be inserted obliquely into the tendinous tissue, and the injection forced in with the same precautions. The pressure may, if necessary, be raised somewhat higher, for, owing to the firmness of the tissue, there is less liability to the occurrence of extravasations.

For displaying these injected preparations they may, if injected with Berlin blue, be first placed in spirit to remove all water and precipitate the colouring matter in the vessels (the process being completed by putting the injected part into absolute alcohol), after which the preparation may be placed in turpentine and mounted in dammar varnish. Another method, and one which succeeds very well, especially with the alkanet injection, is to stretch the injected aponeurosis over a ring of cork and allow it slowly to dry by exposure to the air. When completely dry the injected part may be at once mounted in dammar or in glycerine. By this mode of proceeding the injection is, as pointed out by Bowditch, rendered more complete, for the fluid which may have been extravasated in the interstices of the tissue is apt to become drawn into the lymphatic vessels to supply the place of the watery fluid which becomes lost by evaporation.

Although in many cases it is better to use an apparatus of the kind above described, which enables the pressure which is being employed to be exactly estimated, yet it may be stated that with a little experience the lymphatic vessels, especially those in the firmer tissues and organs, may often be injected with great success by using simply an ordinary Pravaz subcutaneous syringe provided with a fine cannula, driving the injecting fluid into the tissue merely by the pressure of the finger. It is true that extravasations are very apt to be

produced opposite the point of the cannula, but these can often be utilised in the manner before mentioned by gently pressing on them and endeavouring to induce the injection to pass into the lymphatic vessels.

In rare cases a vein is pierced by the cannula, and the system of blood capillaries of the part is then apt to become filled, but both the vessels themselves, and the meshes they form, are much smaller than the lymphatic capillaries, and a knowledge of their general appearance and mode of arrangement in the particular tissue will prevent any error from arising in this way.

Preparation 8.—The lymphatics of the diaphragm may be injected with Berlin blue during life:—A young rabbit is chosen, and enough chloral hydrate is injected under its skin to anæsthetise it completely (about 8 minims of a 50 per cent. solution will suffice). The skin of the belly is then cut through for a couple of inches close to the ensiform cartilage, and the edges are held aside by an assistant, and the muscular wall having been pinched up a blunt-pointed cannula is passed obliquely into the cavity of the peritoneum at its upper part, due care being taken to avoid the liver and stomach. About five cubic centimetres of a saturated solution of Berlin blue, previously warmed, are now injected through the cannula, which is then withdrawn and the animal put aside in a warm place. After four hours, during the whole of which time it remains under the influence of the chloral, it is killed by bleeding. The abdomen is then opened, and the viscera having been drawn aside, the under surface of the diaphragm is exposed, and the blue which covers it is washed off by a stream of water. If the experiment has been successful, it will be found that the whole network of lymphatics of the central tendon is filled with the blue fluid; for this, assisted by the constant respiratory movements of the diaphragm, has passed from the peritoneal cavity directly through the open stomata into the lymphatic vessels. The tendon may be cut out and placed in alcohol,

and eventually, after passing through turpentine, mounted whole in Canada balsam, between two glass plates, and used for examination with a low power of the microscope.

THE SYNOVIAL MEMBRANES.

These structures, which are to be regarded as free surfaces of the ordinary areolar tissue, and present no essential differences in structural appearance from this, may be prepared for microscopical examination by the same methods. The preparations which are of greatest value are those stained with nitrate of silver.

Preparations 9, 10, 11.—Since, as is always the case with the silver method, the parts should be as fresh as possible, and since, moreover, it is convenient to have large joints to work with, a neat's foot should be procured from the butcher's, unless a freshly amputated limb is available. In the neat's foot all three kinds of synovial membranes may be found and prepared. The mode of silvering the synovial bursæ is quite simple, and need not here be detailed; the preparation of the vaginal synovial membranes was described under Connective Tissue (p. 65), this being taken as typical of the structure of that tissue; and the preparation of the synovial surfaces of the joints was given under Articular Cartilage (p. 77). But in the last-mentioned place nothing was said as to the mode of demonstrating the synovial membrane proper, for we had there to do only with the cells and cell spaces of the cartilage, and the transitions met with to those of the connective tissue of the membrane. The appearance presented by the membrane itself is shown in surface sections made in a similar way, but from the inner surface of the capsule of the joint. It will be seen that in the ox the cells, or rather in the silvered preparations the white cell-spaces, form a close irregular network by the union of their processes; in fact, so completely have the cells become extended into branches that it is difficult in many cases to make out where the body of the cell has been. In the human synovial membranes this is not the case; in

fact, the appearances are quite characteristic of ordinary areolar tissue. The arrangement of the corpuscles into epithelioid patches is not infrequent, but there is no continuous epithelioid covering, as in the serous membranes. Moreover, the lymphatics, which were so numerous in those, are not to be seen in the synovial membranes, although blood capillaries are present and in many places approach close to the surface.

Preparation 12.—But to study the characteristic arrangement of the bloodvessels of the synovial membranes and to show the *circulus articuli vasculosus*, a preparation must be made from one of the joints of a limb that has been fully injected. Surface sections are then made of the transitional region where the synovial membrane terminates on the cartilage, and including also a part of the membrane. They are mounted in the usual way in dammar varnish, but without being stained.

Preparation 13.—Finally, the Haversian fringes, with their secondary processes, may be prepared. They may be examined fresh in salt solution, and may also be obtained from the joint which was stained with nitrate of silver. To find them it is best to immerse the joint in fluid, for by this means they are floated up, and may then be snipped off and mounted.

THE LYMPHATIC GLANDS.

Preparation 14.—These are chiefly studied by means of sections. They are hardened in strong spirit, into which they are put immediately after removal from the animal; in two or three days they are sufficiently firm to cut, but improve if left longer. The lymphatic glands of the dog may be recommended for demonstrating the structure of these organs, especially for showing the lymph-sinuses and the cortical nodules. The gland is split after hardening into two equal parts by a longitudinal cut through the hilus, and one of these halves is embedded in wax-mass in the usual way (pp. 124, 125), and with the artificial surface near one end of

the wax cake. The sections, which *must* be very thin indeed, and should include both cortical and medullary substance, are transferred from the spirit to water and thence to a slide, and mounted in glycerine, without staining. If the lymph-paths appear filled up with lymph corpuscles, so that the retiform tissue which traverses them is not well seen, but the whole section appears more or less uniform in structure, these corpuscles may often be in great measure removed by vigorously shaking up the sections with water in a test-tube, or by gently brushing them under spirit with a soft camel-hair pencil. Unfortunately both these methods tend to break up the sections, and indeed it is not necessary to employ them, if the sections are made sufficiently thin.

These organs are amongst the most difficult to demonstrate the structure of satisfactorily, so that it may be well to defer their preparation until some practice has been obtained in making sections of other parts.

CHAPTER X.

THE SKIN, HAIRS, AND NAILS.

Preparations 1–6. Sections of the skin.—Portions should be selected for examination from different parts of the body; the palms of the fingers or toes, the scalp, and a piece from some part of the general surface; *e.g.* the extensor surface of the forearm. The skin of the scrotum may also be prepared, to show the bundles of plain muscular tissue in the subcutaneous tissue or dartos, and a small piece of the ala of the nose, for the sake of the well-marked sebaceous glands, which open into the follicles of the minute hairs found in this situation.

The following method of hardening the tissue can be recommended:—A small piece only is removed, being obtained with as little of the subcutaneous tissue as possible adhering to it. At the same time, if it is desired to examine the larger sweat-glands, this tissue must not be removed too freely, since those bodies extend down into it. It will be found that the fresh skin has a tendency to curl in at the edges; this should be prevented by pinning the piece out on a piece of cork. The latter is then inverted into a beaker containing a mixture of spirit and chromic acid (equal parts of spirit and of half per cent. solution of chromic acid). Here, while the chromic acid hardens the tissue pretty uniformly, the spirit tends to prevent the epidermis from breaking away, as it is apt to do when placed in chromic acid alone. (Simple hardening in spirit also answers in many cases fairly well.) After having been a fortnight in this mixture, the piece of skin is

transferred to strong spirit, and after two or three days in this is ready for embedding and making sections. The piece from the palm of the finger is to be cut in two directions, viz. (1) across and (2) parallel with the ridges formed by the papillæ. It will be easier to cut a thin section if the razor is made to travel from the corium towards the epidermis, rather than in the opposite direction. The pieces of skin which contain hairs should be so embedded as to carry the plane of the vertical section in the direction of inclination of the hairs, so as to gain a view of the hair follicles along their whole extent, and to show the *erectores pili* if possible.

The sections are in each case to be placed in logwood solution, and when sufficiently stained to be washed in water and transferred successively to spirit, oil of cloves, and dammar in the usual way.

The preparations may be much improved, so far as the exhibition of the epidermis is concerned, by being placed in a saturated solution of picric acid for about half an hour before being stained with logwood. In this way the horny parts acquire a bright yellow colour, which contrasts strongly with the violet staining of the Malpighian layer. Owing to the number of nuclei which become stained in it, this part of the epidermis is much darker than the papillary part of the corium which is in contact with it. In the papillæ of the skin of the finger the tactile corpuscles may be sought for. They are generally situated quite near the apex of a papilla, and to see them well it is important to cut the papillæ exactly vertically, so as to include their length. In sections which have been made somewhat obliquely, and in sections cut parallel instead of vertical to the surface, the transversely or obliquely cut papillæ appear as round or oval islands in the midst of the deeper cells of the epidermis. The dentated appearance presented by most of the cells of the Malpighian layer can readily be seen with a high power immersion objective.

Of the sections made from the skin of the finger one or

two of the thinnest should be mounted in glycerine as soon as cut, without staining, merely placing them first in water, to remove the spirit. The fibrous-looking tactile corpuscles can generally be made out better in these than in the preparations which have been mounted in dammar.

Preparation 7.—To show the arrangement of the blood-vessels, sections of skin from a limb which has been minutely injected may be made and mounted in dammar by the usual process. These must either be left entirely unstained or the staining must be very slight indeed. These sections will generally include clusters of fat-cells, with their vessels.

Preparation 8.—The following method will serve for the demonstration of some points in the structure of the corium, as well as the arrangement of the blood-vessels.[1] One of the limbs of an animal, preferably of a dog, is injected with a solution of Berlin blue, the injecting cannula being placed in the principal artery of the leg, and a band being tightened firmly over the upper part of the limb after the injection has been flowing for a minute or two, so as to compress everything except that vessel and prevent the further escape of the injection from the veins of the limb. A pressure of from 4 to 8 inches of mercury is then maintained for several hours. In this way all the vessels become completely distended with the blue fluid, the watery part of which in large measure exudes, so as to render all the tissues œdematous. If coloured gelatine is employed the limb is all the while kept thoroughly warm by placing it over a large water-bath and covering it with a glass plate or bell-jar; and the gelatine in the injecting-bottle and supply-tube must also be kept fluid by similar means. After the time mentioned the artery may be tied and the limb, if gelatine has been employed, removed to a cool place. When it is considered that the gelatine is entirely set, a piece of the skin is cut out, stretched over a dialyzing glass and placed in a beaker of digestive fluid, which has been previously prepared by adding a few drops of a glycerine extract of the gastric mucous membrane to 500 c.c. of a 0·2 per cent. solution of hydrochloric acid. It is then maintained for five or six hours at a temperature of 38° C., when the piece of skin is removed and placed in water for twenty-four hours. Sections

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[1] W. Stirling, in 'Journal of Anatomy and Physiology,' vol. x. pp. 185 and 465.

may then be cut in any desired direction, stained with logwood, and mounted in glycerine; and although not obtainable very thin, yet, owing to their clearness and transparency, the arrangement of the elastic fibres, of the bloodvessels, and of the little muscles attached to the hairs can be traced with comparative facility.

Preparation 9. Hairs.—To examine a hair, all that is necessary is to place it on a slide in a drop of water, cover with a thin glass, and examine with a moderately high magnifying power. By careful focussing the cuticular scales can often be made out on the surface and at the edges of the hair, especially on the small hairs of the general surface of the body. The medulla is often absent in hairs of the head, but may generally be found in those of the beard and whiskers. Many of the black particles which are seen in a hair by reflected light, and especially in the medulla, are merely small globules of air in the interstices of the tissue. That this is so may be proved by cutting off the light which comes from the mirror of the microscope and viewing the object by reflected light, only a moderate power being used. The black particles, if really due to the presence of air, will then appear silvery white, just as in the parallel case of the air which fills the lacunæ in a section of hard bone.

It will be useful to compare the appearances presented by human hair with those exhibited by the hairs of some of the common domestic animals. These are many of them characterised by the regular arrangement of the medulla (this is nearly always present in the hairs of quadrupeds), which forms different patterns in different kinds of animals, so that the species to which the hair belongs may often be determined.

Preparation 10.—The fibrous part of a hair can be broken up into its constituent fibres and cells, if it be first steeped for a time in strong sulphuric acid.

Preparation 11.—The relative proportion of the three constituent parts of a hair to one another is best shown in transverse sections.

To obtain sections of hairs, the simplest plan is to tie a number together and dip the bunch into strong gum, and when this has thoroughly soaked in amongst the hairs to remove the bunch and either let the gum dry and harden by exposure to the air or plunge it into spirit containing a little water, by which in a few hours the whole mass is rendered hard. Sections are then made with a sharp scalpel, and are placed on a slide in water and covered. The water dissolves the gum away, and all that is now necessary in order to make the preparation complete is to allow a drop of glycerine to diffuse under the coverglass, which may then be secured as usual.

The hair-follicles and roots of the hairs are seen in the sections of skin.

Preparation 12.—The nails are studied by means of vertical sections made both longitudinally and transversely. The finger (or toe) should if possible be previously injected, and the nail with the matrix and surrounding skin having been removed, may be hardened either in spirit or by the spirit and chromic mixture. When ready for cutting, the piece should first be bisected longitudinally; and one of the halves having been embedded in a wax and oil mixture, with rather an excess of wax, so as to render it harder than usual, one or two longitudinal sections (which need not be very thin) along the whole length, to show the general relation of the nail to its matrix and to the epidermis, are to be made with a sharp strong scalpel. These need not be stained; they may be mounted either in glycerine or, if injected, in dammar; they are intended only for examination with a low power. The other piece may also be embedded in the same mixture, but in such a way that the laminæ, which in the matrix represent the papillæ of the skin, are cut transversely. The sections must be as thin as possible, and stained either with logwood alone or with picric acid and logwood in the same way as was recommended for the sections of skin, before being mounted in dammar.

But, owing to the substance of the nail being so much harder than the subjacent matrix, it is very difficult to get both parts equally thin. They can, however, be got of much the same degree of hardness by means of the gum method. The piece to be cut, which should be quite small, is placed in syrupy solution of gum and left over-night; it is then transferred to a mixture of spirit with one-sixth of its volume of water. After a few hours the gum which has penetrated into the substance of the tissue will be hardened throughout, and the mass can be embedded and cut in the desired direction, the knife being wetted with some of the same spirit-mixture. Strong spirit should not be used, since the gum is entirely dehydrated by this, and becomes so hard as to turn the edge of the knife. Instead of embedding the piece in wax-mass it will be found a cleaner and more convenient method to make a slit in a winebottle cork, insert the hardened gum-impregnated tissue in the proper position in the slit, and maintain it in place whilst cutting the sections by the pressure of the finger and thumb. The sections are transferred from the spirit to water, which dissolves out the gum; when quite free from this they are stained and mounted as before.

It will be well, before commencing the description of the mode of preparing the several viscera for microscopical examination, to revert to one or two preparations which were purposely deferred until the modes of preparing sections and of injecting the bloodvessels had been explained.

Injected muscular tissue is obtained from any injected limb, and should be hardened in strong alcohol. If the piece is not large enough to hold in the fingers, it must be embedded, and moderately thin sections made both longitudinally and transversely, and mounted, unstained, in dammar.

Transverse sections of a nerve-trunk may be made from any large nerve that has been hardened in picric acid (forty-

eight hours), and subsequently in alcohol. The sections are to be stained with picro-carmine (twenty-four hours), and may be mounted in glycerine. By this method the medullary sheath of the nerves and the elastic tissue of the perineurium and epineurium are stained yellow, the connective tissue lamellæ and bundles pale red, and the corpuscles and nuclei and the axis cylinders a darker red.

Longitudinal sections of a nerve-trunk from an injected limb may be prepared in the same way as those of injected muscle.

The lymph spaces which lie between the lamellæ of the perineurium of the nerves, and extend also amongst the fibres within the funiculi, can very readily be injected with 2 per cent. solution of Berlin blue by merely sticking a very fine injecting cannula into a funiculus, and employing moderate pressure. The injection runs along the course of the nerve almost as freely as if it were an open tube. A piece of nerve which has been injected in this way is to be cut out, hardened in spirit, and transverse sections prepared and mounted in dammar, without staining, to show the course taken by the injection. At the entrance of the nerve-roots into the spinal canal the perineural clefts communicate with the sub-arachnoid space, so that the nerves can be injected by merely forcing the injecting fluid into this.

Longitudinal sections of ganglia, both spinal and sympathetic, may be prepared from specimens that have been hardened either in picric acid and spirit or in 2 per cent. bichromate of ammonia solution for five days, then in weak spirit (half water) for twenty-four hours, and then in strong spirit. The sections are to be stained in logwood, and mounted in dammar.

The sections of developing bone (p. 91) (instructions for embedding which, by the cacao-butter process, were previously given) may also be now prepared.

CHAPTER XI.

THE HEART.

Preparation 1. The cardiac pericardium.—The pericardium which covers the surface of the heart is prepared by the same methods as other serous membranes. Of these the only one which need here be described is that by nitrate of silver. This is as follows: In an animal which has just been killed the thorax is freely opened, and the pericardium having been torn open, the base of the heart is secured by a tape ligature, the great vessels being then cut beyond the ligature, and the organ removed without allowing its surface to be smeared with blood. A part of the surface is now brushed firmly with a soft camel-hair brush moistened with distilled water, with the object of removing the superficial layer of epithelioid cells. The heart is next dipped for a moment in distilled water, and then nitrate of silver solution is poured over the whole surface, and allowed to be on it for three minutes, after which the organ is rinsed again in distilled water, and finally placed in spirit, in the sunlight. When sufficiently browned it is removed from the window, and left for some hours until the surface is hardened by the alcohol. Surface sections are then made of both unbrushed and brushed parts, and after soaking in water for a minute or two are mounted separately in glycerine. The unbrushed specimens will show the epithelioid layer; the brushed ones should exhibit the subjacent connective tissue, with its cell-spaces, lymphatics, bloodvessels, and nerves.

Preparation 2.—The muscular substance of the heart is studied in teased preparations and in sections. For the teased preparations the heart of a young animal should be chosen, since in these the fibres separate more readily into their constituent cells. A very small shred is placed in a comparatively large quantity of $\frac{1}{10}$ per cent. osmic acid for ten days or a fortnight; it is then broken up in water as minutely as possible, and the preparation covered and scanned with a high power. Numerous little fragments of varying shapes will be found scattered over the preparation. On careful examination it will be apparent that each possesses a nucleus, which can be made more conspicuous by allowing a little dilute logwood solution to run underneath the coverglass. These little fragments of the cardiac muscular tissue, which have the characteristic indistinct striation of that substance, are the cells which by their union end to end form the fibres.

Preparation 3.—To show the arrangement of the fibres, and the interstitial tissue and vessels, a piece of the muscular substance is to be placed in strong spirit. In two or three days it will be firm enough to cut. Sections are to be made both parallel with and across the direction of the fibres; they are to be stained with logwood and mounted in dammar.

Preparation 4. The endocardium.—To display the endocardium the silver method again comes into requisition. That part of the lining membrane covering the septal wall of the right ventricle is the best to prepare, on account of its relative smoothness. The right ventricle is opened in a fresh heart, and the outer wall removed entirely, and then a large piece of the smoothest part of the exposed surface of the septum is sliced off with a razor. A part only of the endocardium of the detached piece is brushed, as in the case of the pericardium, and the whole is then washed and treated with silver solution. After three minutes it is put into spirit as before, and when browned and hardened surface sections are cut and mounted in glycerine.

Preparation 5.—In addition to these silvered preparations, the endocardium should be examined in the fresh state. This is done by dissecting off a piece of the membrane in salt solution and examining it both with and without the addition of acetic acid. Other portions may be teased out with a view to the demonstration of the elastic and muscular tissue. The methods for making and preserving these preparations are the same as were employed for showing the structure of the coats of the bloodvessels, to the description of which the student is referred (p. 121). It may be noted that in some animals—the sheep, for instance—the peculiar, large, cubical or oblong cells which form, in series, the fibres of Purkinje will be found in the endocardium. They are about the size of fat-vesicles, which are also found in the endocardium in this animal, but the two could hardly be confounded, for the cells forming Purkinje's fibres have a clear or slightly granular nucleated central portion which does *not* strongly refract the light, and a striated circumference, which is apparently continued into that of the neighbouring cells; whereas the fat-cells, although they may also occur in rows, and may be of much the same size as the cells in question, present, by virtue of their strong refracting power on light, a totally distinct appearance.

Preparation 6. Lymphatic system of the heart.—If the fine cannula of a Pravaz syringe filled with Berlin blue solution is stuck into the muscular substance of the fresh heart at any part, and the fluid is forced out at the point, the injection will pass freely into the lymphatic interstices between the muscular fibres, and if the tube is inserted near the outer or inner surface, will find its way into the lymphatics of the pericardium or endocardium, which can in this way be readily displayed.

CHAPTER XII.

THE LUNGS.

Preparations 1–2. The pulmonary pleura.—The serous membrane which covers the surface of the lungs as well as that which lines the wall of the thorax is prepared by the silver process. For the pulmonary pleura, the lungs of a small animal that has just been killed are to be removed entire, and moderately distended with air through the windpipe, the bronchi being then tied and the two lungs separated. One is rinsed for a moment in distilled water, and a little nitrate of silver solution is allowed to flow over the surface; after the lapse of a minute this is washed off again with distilled water, and the organ is then immersed in a beaker of spirit and exposed to the light.

The surface of the other lung is to be firmly brushed with a wet camel-hair pencil, to remove the epithelioid cells of the surface before treating it with the silver solution. This may be suffered to remain a minute or two longer in contact with it than with the other lung; in other respects the treatment is similar. Both preparations are left in the light until they appear sufficiently stained, after which they are to be placed on one side in the spirit for twenty-four hours. They will then be sufficiently hard to render it possible to shave off a thin slice from the surface. The sections so made are to be placed in water, and subsequently mounted in glycerine, with the outer surface uppermost.

Preparation 3.—The costal pleura is to be prepared *in*

situ after the removal of the lungs and heart. That of one side may be brushed, the other not; on the latter the silver solution is, as before, to be allowed to remain a shorter time than on the brushed part, where the fluid has to penetrate into the lymphatic vessels, and into the substance of the tissue. The whole thorax may then be cut off from the rest of the trunk and exposed under water to the light; or, if it be too large to do this conveniently, a piece only of the thoracic parietes on each side is to be removed and pinned out on to a loaded cork, which is then placed in a dish of water in the sunlight. When stained pieces of the membrane must be carefully dissected off, without pulling upon or injuring the tissue in any way, floated upon a slide, the excess of water poured off or soaked up with blotting-paper, all creases removed from the membrane, and finally the cover-glass superadded, with a drop of glycerine.

Preparation 4. The lung tissue.—The structure of the lungs themselves is best shown by means of sections. The tissue is hardened in the following way:—

The organs having been removed from the chest of a recently killed rabbit or cat, care being taken not to scratch their surface with the broken ends of the ribs, a glass cannula is tied into the end of the trachea (or into either bronchus). The cannula is then connected by an indiarubber tube with an injection bottle, which is filled with a weak solution of chromic acid ($\frac{1}{6}$ per cent.). By blowing air into the tube this solution is made to flow into the lungs so as to distend them moderately. The trachea or bronchus is now tied up, the cannula removed, and the lungs are immersed in a large quantity of a solution of chromic acid of similar strength. After two days the fluid is changed, $\frac{1}{4}$ per cent. solution being substituted, and the organs are cut into pieces, to enable the fresh fluid more readily to penetrate. After a week more in this the pieces are placed first for twenty-four hours in weak spirit and then in strong spirit.

The bits, though small, will probably be large enough to

hold in the hand and cut without embedding. Sections may be made both across and along the course of the bronchial tubes, stained with logwood (it will be found that the sections must be left for a considerable time in logwood, for they stain with difficulty), and, after going through the usual processes, mounted in dammar.

Preparation 5.—But, owing to its spongy nature, it will be found almost impossible to cut very thin sections unless the interstices of the tissue are filled with some firm material, and the following directions may accordingly be observed, if thinner sections than can be obtained in the ordinary way are desired:—A small piece only (not larger than a kidney-bean) of the lung hardened as above described is placed for two days in alcoholic solution of logwood Kleinenberg's.[1] The piece of tissue will have been stained throughout of an intense dark violet colour, and will look almost black. On removal from the staining fluid it is transferred through alcohol to oil of cloves. After an hour in this, by which time the oil will have had time to penetrate its whole thickness, it is put into melted cacao-butter, which is kept in the fluid condition by a temperature of not more than 42° C., and allowed to lie in this for four hours. An oblong cake of the cacao-butter having been previously made by pouring some of the melted fat into a paper mould, a little pit is scooped in it near one end, and the piece of tissue, now soaked through and through with the cacao-butter, is placed in the pit in a

[1] Kleinenberg's solution is made in the following way (Foster and Balfour):—

(1.) Make a saturated solution of crystallised calcium chloride in 70 per cent. alcohol, and add alum to saturation. (2.) Make also a saturated solution of alum in 70 per cent. alcohol. Add (1) to (2) in the proportion of 1 : 8. To the mixture add a few drops of a saturated solution of hæmatoxylin in absolute alcohol.

This solution may be used in very many cases for staining sections, in place of the ordinary watery solution of logwood alum. It may, if required, be diluted with the mixture of 1 and 2. The stained sections are placed at once in strong spirit.

position convenient for making sections, and the pit is then filled with the melted cacao-butter. This as it hardens adheres firmly to and sets into one piece with the cake. When quite hard—a process which takes a considerable time—sections of the tissue are made with a razor wetted with spirit. Since all the cavities are filled by and the tissue is thoroughly impregnated with the cacao-butter, the whole cuts like a homogeneous piece of this substance, and sections can be made as thin as desired. As they are cut they are placed in oil of cloves, which removes the cacao-butter in a few minutes, even in the cold; but in the winter season it is better to accelerate the solution by slight warmth. The sections can then be mounted in dammar.

These stained sections of lung may be first examined with a moderate power, but afterwards a power of 400 or 500 diameters should be employed, in order to see the details of structure; the ciliated epithelium, muscular layer, and cartilaginous plates of the bronchial tubes, with the mucous glands, nerves, lymphatics (seen in section as mere clefts), and patches of lymphoid tissue in their walls; the branches of the pulmonary artery accompanying them; the mode in which the terminal air-tubes dilate into the infundibula; the air-cells or alveoli, almost covered with a network of capillaries, which are seen also on the septa between the alveoli, projecting first into one and then into the other of two neighbouring air-cells. Where they run vertically the capillaries appear in optical section as circular spots, looking not unlike nucleated cells. But the excessively delicate epithelium of the air-cells cannot be well seen in these preparations, for the epithelium cells remain almost unstained, and it is not easy to differentiate their nuclei from those of the closely subjacent capillaries.

Preparation 6. Epithelium lining the air-cells.—In order to demonstrate the epithelium cells we make use of the nitrate of silver, but the mode of proceeding is somewhat different from that ordinarily employed. A gelatine mixture is made by taking ten grammes of gelatine, and, after

soaking it in cold distilled water, melting it, and adding if necessary more warm distilled water until the mixture measures 100 c.c. A decigramme of nitrate of silver is dissolved in a little distilled water and added to the gelatine, and the mixture is transferred to a glass syringe, which is kept warm over a water-bath. An animal—preferably a young one—having been killed, the lungs are removed, a glass cannula with a small piece of indiarubber tube attached is tied into the trachea, and then the point of the syringe is slipped into the open end of the indiarubber tube, and enough of the gelatine mixture injected into the lungs to distend them pretty completely. The trachea is now tied and the cannula removed from it. The lungs are then put aside into a cold place until the gelatine within them has fully set, when sections, which should be as thin as possible, are made with a razor, either not wetted at all or with distilled water only. The sections so obtained are placed on a slide in glycerine, covered, and exposed to the light. As soon as they seem sufficiently stained they may be examined with as high a power as possible, for the purpose of making out the silver-lines between the epithelium cells.

Preparation 7. Bloodvessels of the lungs.—Lastly, the pulmonary vessels are to be injected and sections made of the injected lung. The red gelatine injection may be used; this and everything else is to be got ready just in the same way as for the injection of the aortic system, but a syringe filled with melted cacao-butter must be connected with the trachea in the same way as for the gelatine mixture in the last paragraph; and moreover the arterial cannula is of course to be passed through the *right* ventricle and tied into the pulmonary artery, instead of into the aorta. Immediately the gelatine injection has been set flowing through the pulmonary vessels the lungs are pretty fully distended with the cacao-butter; the pressure is then raised in the injecting apparatus to about four inches of mercury. The left ventricle is first slit, to let the blood out of the pulmonary system, and then

clamped, to prevent the escape of the injecting fluid, which is allowed to pass in for a few minutes until it is thought that the vessels must all be completely filled; this can be partly made out from the colour which the lungs assume. The trachea and the base of the heart are then ligatured, and the whole is left for some time to cool, so that both gelatine and cacao-butter are fully set. The lungs are then cut out and placed in weak spirit; after a day they are transferred to strong spirit, and in another day or two sections may be made (mostly vertically to the surface of the lung), placed in warm oil of cloves, to dissolve out the cacao-butter, and then mounted in dammar. If it is desired to stain the tissue somewhat, so as to show the general structure of the lung as well as the arrangement of the bloodvessels in the same preparation, this can be done by placing the sections, after the cacao-butter has been dissolved out of them, first in absolute alcohol, to get rid of the oil of cloves, and then for a few minutes in Kleinenberg's logwood until they are sufficiently coloured. The transference through absolute alcohol and oil of cloves into dammar is then proceeded with, as in the case of other sections.

Preparation 8. Larynx and trachea.—The trachea and larynx are hardened in $\frac{1}{6}$ per cent. chromic acid (ten days), the hardening being completed by spirit, and the sections, which may be longitudinal of the cartilaginous part and transverse of the posterior membranous part, are to be stained with logwood (they will require a considerable time), and mounted in dammar. It will, of course, be necessary to embed the tissue, and it will be found advantageous in cutting to pass from the mucous membrane outwards, instead of *vice versâ*. But, owing to the great difference in hardness between the cartilaginous rings and the rest of the tissue, it is difficult to get a complete section equally thin throughout.

Preparations 9–10. Bloodvessels and lymphatics of trachea.—In addition to these sections flat preparations showing the bloodvessels, and others showing the lymphatics

of the mucous membrane, may be made. The former are got from any animal that has been injected entire, the mucous membrane being dissected off and mounted in dammar. The lymphatics are readily filled by the puncture method by sticking the point of the injecting cannula into the mucous membrane, and forcing a little Berlin blue or alkanet-turpentine in. It will hardly ever fail in finding its way into the numerous lymphatics of the mucous membrane. The injected portion is dissected off and mounted in dammar varnish or in glycerine.

Teased-out bichromate of potash preparations to show the separated epithelial cells have already been made (p. 52).

Preparations 11–13. Ductless glands of the larynx and trachea.—The thyroid and thymus are studied chiefly by means of sections, for facilitating the preparation of which the glands are hardened in alcohol. They should not be put entire into this, but either cut into pieces or deep cuts should be made into their substance, so that the preservative fluid may penetrate rapidly. It is well to place them at first in weak spirit (half water) for twenty-four hours, and then to transfer them to the strongest possible, in which they are allowed to remain for a few days until hard enough to cut thin sections from. Like most organs which have been hardened in alcohol alone, they stain very readily. The so-called 'colloid' which is met with in the vesicles of the thyroid is coloured by the logwood. Both glands may be advantageously obtained from the body of a newborn child, which should if possible be injected, so that the mode of distribution of the bloodvessels, especially those of the thymus, may be displayed.

The concentric corpuscles of Hassall which are met with in the thymus can be seen in sections of that organ, but may also be studied isolated in preparations of the fresh part teased-out in salt solution.

CHAPTER XIII.

THE MOUTH AND PHARYNX.

Mucous membrane of the mouth.—Portions of the lining membrane of the mouth are best prepared in the way recommended for the skin, viz. by being pinned out upon a cork and immersed for two or three days in a mixture of equal parts of spirit and half per cent. chromic acid solution. Sections of the cheek or lip may also thus be readily prepared.

THE TEETH.

Preparation 1. Sections of hard tooth.—No preparations exhibit the structure of the teeth better than these. The hard tooth is ground down first on one side and then on the other, until a thin section only remains, and this is mounted in hard Canada balsam in such a way that the air still remains in the dentinal tubules, the lacunæ of the cement, the interglobular spaces, and other minute cavities that may be present. The preparation is similar to that of bone, but presents greater difficulty. Such specimens may advantageously be purchased, for their preparation involves the expenditure of a large amount of time and labour; unless the use of a lapidary's wheel can be obtained, when the process is much facilitated. They should in every case be studied first with a low power, and afterwards with a high power objective.

Preparation 2. Sections of softened tooth.—But, in ad-

dition to the facts which the hard specimens will show, various others may be made out in teeth which have been softened by immersion in an acid.

The acid generally used when the structure of the (decalcified) dentinal substance only is to be investigated is the hydrochloric. A 10 per cent. solution may be employed, and the tooth is steeped in this until entirely soft, after which it may be preserved in spirit. Sections are to be made in planes both parallel with and across the direction of the dentinal tubules.

Preparation 3. Dentinal sheaths.—To show the sheaths which line the dentinal tubules, a piece of such softened tooth is transferred to strong hydrochloric acid (contained in a watchglass, which is covered by another, inverted). In this it may be left for about an hour, after which time all that will be found is a tenacious soft mass occupying its place. If some of this be removed with a small pointed piece of wood, placed on a slide, covered, and examined, it will be found to consist wholly of fine tubular threads—the dentinal sheaths. These being composed of a substance which resists the solvent action of strong hydrochloric acid longer than the other animal tissues, remain for a time visible after the rest of the dentinal substance has disappeared.

Preparation 4. Soft tissues of the teeth.—But to study the soft tissues—that is to say, the pulp and odontoblasts with the processes which these send into the dentinal tubules—we must, as in the parallel case of bone, employ a reagent which, whilst softening the hard parts, at the same time preserves and hardens the soft parts. Of these picric acid is the best to employ. The freshly-extracted tooth is placed in a saturated solution of the acid, and crystals of the latter are from time to time added as required; the solution being stirred as frequently as possible with a glass rod. It is well to break the tooth first, so as to expose the pulp cavity, if this can be done without disarranging the contents too much. When softened throughout—this can be tested by attempting

to pass a fine needle through it—the tooth is placed in spirit, which should be changed day after day until it ceases to become much coloured by the excess of picric acid. All that remains to be done is to cut the tooth in halves vertically, and embedding one of the halves in a hard wax-mass, cut thin slices from the artificial section, stain these with log-wood, and mount them in glycerine.

Preparation 5. Study of the teeth 'in situ.'—Still more instructive preparations are obtained by softening a portion of the lower jaw with the teeth *in situ*, and making sections through the whole structure. It is best to take the jaw of a small animal—a rat, for instance. The flesh having been cleared away, the softening is effected with picric acid in the way above described; and then, after due immersion in spirit, the piece is embedded, cut, stained with logwood, and mounted in glycerine as before.

Besides showing the teeth and the way in which they are inserted into the lower jaw, the structure of this bone is itself well demonstrated. At the lower part the constantly growing incisor, which extends in the rat below the molars to the back part of the jaw, exhibits the large elongated odontoblasts of a developing tooth, with their well-marked dentinal processes (fibres of Lent), which in some parts project like harp-strings across a small space which intervenes between the cells and the dentinal substance. It will be remarked, also, that in these teeth the most newly-formed layer of dentine becomes, especially near its junction with the older parts, very intensely stained by the logwood. This is the case with all teeth which are still in process of development. Carmine has not the same action.

Preparation 6. Development of the teeth.—For the study of the development of the teeth sections are made of the jaws of embryos and young animals. Perhaps the most convenient to choose are newborn rats, since sections of their jaws exhibit not only the mode of development of the teeth, but also that of the hair, the bone of the lower jaw

(which ossifies in the connective tissue around Meckel's cartilage), the tongue, and many other parts. The preparation is as follows:—The fœtuses or young animals are decapitated, and the heads dropped into a large beaker of $\frac{1}{6}$ per cent. chromic acid. After a week's time, during which the liquid is now and then stirred, they are transferred to weak spirit, and in twenty-four hours to strong spirit. After being in this a day or two they are ready for cutting. Either the lower jaw is removed and embedded separately, or the whole head is placed in the mould, and both jaws are cut simultaneously. The sections are to be stained, some with logwood, some with carmine solution (made by dissolving 2 grammes of carmine in a few drops of ammonia, and diluting with water to 100 cub. cent.). The stay in chromic acid may not have been long enough to remove all the earth from the partly developed bones and teeth, but what still remains is so small in amount that it will not prevent a thin section being made. The earlier stages in the development of the teeth may be perhaps seen in the molar region; the later stages comprising the development of the dental tissues, especially the dentine and enamel, may be studied in the much more advanced incisors, which, as just pointed out, extend backwards in these animals through the greater part of the length of the jaw.

THE TONGUE.

Preparation 7.—Small portions of this organ from different parts are hardened in 2 per cent. bichromate of potash (fourteen days), and subsequently in spirit, and are embedded, so as to cut vertically to the surface of the mucous membrane. The sections are stained with logwood, and mounted in dammar varnish. A double staining with picric acid and logwood may also be employed in the same way as with the sections of skin (p. 167). The stratified epithelium, the papillæ of the mucous membrane, and the arrangement of

the muscular fibres as well as the mode of termination of the superficial muscular fibres in the connective tissue of the mucous membrane may be studied in these sections. Some of the sections include mucous glands, which differ in appearance according to their condition at the time of death. If they had not been recently stimulated by the ingestion of food or otherwise, the cells of the gland will still be filled with mucus, as shown by their swollen appearance, and by their becoming strongly stained by the logwood; whereas, on the contrary, if they have recently discharged their secretion, the cells will be granular and almost colourless, and there will be indications of mucus in the lumina of the ducts.

Besides the mucous glands others may be seen in the neighbourhood of the papillæ vallatæ, which secrete no mucus, and consequently do not present the above differences of staining. Their ducts, if the section pass in the direction they take, will be found to open almost without exception into the fossæ of the circumvallate papillæ, or, at all events, near those parts in which taste-buds have been found. The taste-buds themselves may possibly be seen in the epithelium on the sides of the papillæ vallatæ, and also in that which covers the mucous membrane near the root of the tongue on each side. The description of other modes of preparing them will, however, be deferred until the organs of special sense are treated of.

Preparation 8. Vessels of the tongue.—Sections should also be made of an injected tongue. These will show not only the numerous vascular loops in the more obvious papillæ of the mucous membrane, corresponding in number with the microscopic secondary papillæ, but also the arrangement of the vessels in the muscular substance of the organ. The injected specimens are much improved by slightly staining them with logwood.

Preparation 9. Palate and tonsils.—The soft palate and the tonsils may be hardened in the same way as the tongue, or,

preferably, by immersion for a week in $\frac{1}{6}$ per cent. chromic acid solution, and subsequent placing in spirit. The sections are stained with logwood and mounted in dammar.

THE SALIVARY GLANDS.

Preparation 10.—These organs are prepared by placing small pieces of them as soon after death as possible in a mixture of spirit and $\frac{1}{2}$ per cent. chromic acid solution, equal parts of each. After two or three days they are transferred to spirit, and in a day or two more will be ready to cut. They may also be prepared by merely being placed in strong spirit for three or four days. The embedding, staining with logwood and mounting, are effected in the ordinary manner, the chief difficulty being met with in the fact that, owing to the loose way in which the lobules are held together by the intermediate connective tissue, the sections are very apt to become broken up by the agitation which ensues from the mixing of the fluids, particularly when they are transferred from spirit to water or to a watery solution of logwood. An alcoholic logwood (Kleinenberg's) may, however, be used, and in any case it will be found that the small pieces exhibit all the details of structure quite as well as larger ones; thin sections which have thus become broken up need not therefore be rejected.

Preparation 11.—The difference in the structure of the salivary glands previous and subsequent to the state of secretory activity is best studied in the submaxillary of the dog, the animal being killed in the one case after some hours' fasting, in the other a short time after food. The glands are hardened and sections are prepared in the manner above described, and the differences in the appearance of the alveoli in the charged and discharged conditions respectively of the glands are noted.

Preparation 12.—Most of the ordinary hardening solutions (alcohol and chromic acid, for instance) considerably alter the salivary cells, so that in sections of the glands the cells are scarcely ever seen of

their normal form and appearance. Osmic acid preserves them in a more natural condition than most reagents, so that, to study the individual elements, a very small portion may be placed in a 1 per cent. solution of this reagent, and after forty-eight hours broken up finely in a drop of water on a slide. The preparation may be preserved in glycerine.

CHAPTER XIV.

THE ŒSOPHAGUS AND STOMACH.

Preparation 1.—The œsophagus is best hardened for the preparation of sections by a mixture of equal parts of chromic acid ($\frac{1}{2}$ per cent. solution) and spirit. After three or four days in this the tissue may as usual be transferred to spirit. Before putting it into the mixture it should, if but a small piece be employed, be pinned out upon a piece of cork, so as to stretch it slightly and avoid folds. But if a tubular piece be available, this object may be effected more satisfactorily by distending the organ with the preservative solution through a glass cannula tied into one end, the other end having been secured by a ligature before the distension ; the piece is then immersed in the mixture for twenty-four hours, after which it may be cut open. In embedding the gullet and other membranous parts, of which it is desired to obtain sections vertical to the surface, it is well to proceed in a way somewhat different from usual, the transfixion with a pin being discarded. A layer of the melted wax-mass is first poured into the mould so as to fill it nearly half-full; the piece of gullet to be embedded is deprived of all superfluous moisture by placing it on blotting-paper for a few seconds, and then, as soon as the wax-mass in the mould is hard enough to support its weight, it is placed in the desired position near one end of the box, which is filled up with more wax-mass. This, especially if a few degrees above its melting-point, adheres firmly to the portion of wax-mass which was first poured in,

so that the whole forms a uniform cake, with the tissue embedded in it.

Moreover, in embedding a piece of one of the membranous viscera it is well to place it, as a general rule, so that the sections shall be exactly transverse to the axis of the viscus, following, therefore, the direction of the circular muscular fibres and cutting the longitudinal across. When the direction of the section is known, it is easier to understand the appearances which the various parts present when cut. Sections cut parallel to the axis of the viscus, and taking therefore the direction of the longitudinal muscular fibres, may be made with equal advantage, but oblique directions should be avoided.

Preparation 2. Bloodvessels of the gullet.—The arrangement of the vessels is best shown in a flat preparation. A small piece, obtained from an injected animal, is transferred, without staining, from the spirit to oil of cloves, being subsequently mounted in dammar, with the inner surface uppermost. Such a preparation is only useful for examination with a low power, but by this the arrangement of the vessels in the successive strata can be well made out.

Preparation 3. The stomach.—The stomach should always be prepared as soon as possible after death, for, in the first place, the columnar epithelial cells covering the inner surface soon become altered; and secondly, if digestion were proceeding in the organ at the time of death, the mucous membrane itself becomes attacked by the gastric juice in a very short time.

The abdomen, therefore, is to be opened as soon as the animal (a cat or dog) is dead, and the œsophagus cut as near the diaphragm as possible, and the duodenum about two inches beyond the pylorus; the folds of peritoneum connecting the viscus to the liver and neighbouring parts are also severed, and the stomach is removed. If the organ is empty or if the contents are fluid enough to admit of being poured out through the pylorus, it may be prepared as a whole by dis-

tension with the spirit and chromic mixture; spirit alone also answers very well. The duodenal end is tied up and a glass cannula is fastened into the œsophageal end. This is connected by an indiarubber tube with a glass tube which passes to the bottom of a bottle containing the hardening fluid. A second tube passes just through the cork of the bottle, and by blowing through it the fluid is forced into the stomach. When the organ is moderately distended the indiarubber tube is clipped, to prevent any of the liquid being forced back into the bottle by the contraction of the muscular walls of the stomach; the gullet is then secured by a ligature, and the whole organ is immersed in a large bottle or covered beaker filled with the same mixture of chromic acid and spirit. After twenty-four hours it should be opened and put into fresh fluid; or, if it is not desired to keep the whole of the organ, small pieces only from different parts are so transferred. (Indeed, if the stomach be too large to harden as a whole, or difficult to be cleared of its contents, small pieces may be cut out from the fresh organ, pinned out on a piece of cork or cake of wax, and thus immersed in the spirit and chromic mixture.) In two or three days more the tissue is hard enough, if the spirit be of the strongest, to cut sections from, and small pieces may accordingly be embedded with this view. The sections, which should comprise all the coats of the organ, are to be stained with logwood and mounted in dammar as usual. They will show well enough the relative thickness of the several layers and many of the structural points, but for making out distinctly the structure of the mucous membrane and the characters of the cells which occupy the gastric glands it is necessary to make thinner sections than are easily obtainable in conjunction with the muscular coat.

Preparation 4. Gastric glands.—With this object, then, small pieces of the fresh mucous membrane are taken from two distinct parts, one from near the pylorus and the other from the cardiac fundus, and placed at once in absolute alcohol. When hardened they are embedded separately,

and vertical sections, as thin as possible, are made. Those from the pyloric piece are to be stained with logwood and mounted as before in dammar, but one or two of the thinnest may be selected and mounted in glycerine. Those from the cardiac piece are to be stained and prepared in three different ways. In the first place, two or three may be treated as just recommended for those from the pyloric piece. Secondly, other two or three are to be placed for twenty-four hours in a weak solution of carmine (the carmine solution, p. 184, diluted five times). Thirdly, one or two others are to be stained with aniline blue. There are different kinds of this sold in the shops; that known as Nicholson's No. 1 gives good results. A one per cent. solution is used, and the sections are left in for about thirty minutes. They are then placed in a watchglass containing a mixture of glycerine and water, equal parts, and are finally mounted in glycerine. The carmine-stained sections are also mounted in glycerine, after having been rinsed in water.

These different modes of staining bring out distinctly the differences between the various kinds of cells found in the peptic glands. In the logwood preparations the peptic cells will be found stained rather less than the rest, whereas in the sections stained by carmine and aniline they are coloured more deeply. This is especially the case in the aniline preparations, where the peptic cells are stained of a deep blue, whilst the other cells remain almost colourless.

Preparation 5. Horizontal sections.—Besides the vertical sections of the mucous membrane others are to be made parallel to the inner surface, and therefore so as to cut the glands across. To effect this a small piece of each region is embedded, with the part which corresponds to the inner surface of the stomach placed opposite the end of the mould. It is then gradually cut into slices, which will, of course, comprehend in succession first the mouths of the glands, then their necks, and finally the deeper parts. The sections

so obtained are to be stained and mounted in the same way as the vertical sections.

Preparation 6. Cells of the glands, isolated.—In addition to studying them in sections in this way the cells of the glands are also to be studied in teased-out preparations of the fresh mucous membrane. If prepared in serum they will show better than by any other method the characters of the different kinds of cells. The cells will be more readily obtained separate if a small piece of the membrane is placed in $\frac{1}{8}$ per cent. bichromate of potash solution for twenty-four or forty-eight hours, but the cells are apt to be somewhat altered, and the columnar cells of the general surface and mouths of the glands to become transformed, by the swelling and escape of their contained mucus, into goblet-cells.

Preparation 7. Bloodvessels of the stomach.—Both vertical and horizontal sections of an injected stomach are to be made This may be obtained from the animal which was injected entire: if it were a rat, the preparations are to be made from the pyloric half of the organ, since in this animal the cardiac part has a non-glandular mucous membrane with stratified epithelium like that of the gullet. The vertical sections need not be very thin; they are improved by being placed for a few minutes in dilute logwood, so as to become slightly coloured, before being mounted in dammar by the ordinary process. Instead of cutting horizontal sections a small piece of the injected stomach may, if from a small animal, be simply mounted flat with the inner surface uppermost, without staining.

Preparation 8.—An attempt may be made to inject the lymphatics of the gastric mucous membrane with Berlin blue, but the process requires considerable care and experience, since it presents unusual difficulties. Indeed, it is only quite recently that attempts to fill any lymphatics except those in the deepest part of the membrane have been successful. If a successful result is obtained the injected portions are hardened in alcohol, and vertical sections, which may be tolerably thick, made and mounted in dammar.

CHAPTER XV.

THE SMALL AND LARGE INTESTINE.

Preparation 1. Sections of small intestine.—Pieces of the small intestine are to be prepared in exactly the same way as the stomach, the mixture of alcohol and chromic acid solution being employed to distend the gut, which is then immersed in the fluid. After a few hours the intestine is opened and the fluid changed, and in three or four days the tissue is transferred to spirit, to complete the hardening. Three pieces of the small intestine are to be preserved in this way, viz. one from the very commencement of the duodenum (this will probably have been included in the stomach preparation); a second from the jejunum; and the third from the ileum, including one of the patches of Peyer. The pieces may be obtained from a cat, dog, or rabbit, the contents of the intestine being first washed out by forcing a rapid stream of the alcohol and chromic fluid through them before tying up the further end. Instead of distending it with the preservative fluid, the gut may be opened and kept in an extended state by pinning it on a cork or cake of wax, which is then inverted into the fluid.

In embedding the small intestine in wax-mass care should be taken that the inner surface does not retain too much spirit between the villi, for this would prevent the melted wax-mass from penetrating between them, so that they are thus left without a support whilst being cut. At the same time the surface should on no account be allowed to become quite dry.

It is necessary, in order to see the structure of the villi, that the sections should be very thin indeed—so thin, in fact, as to include not the whole thickness of a villus, but only a longitudinal slice; otherwise the epithelium on its surfaces interferes with the view of the internal structure. With a very sharp razor and considerable dexterity, this may be effected even when the intestine is embedded by the ordinary process, but the thin sections so obtained are apt to become broken up during the process of staining and subsequent washing. It is therefore well to adopt for this tissue the cacao-butter process, described at p. 176. A small piece is stained with Kleinenberg's logwood, as there recommended, and after passing through alcohol and oil of cloves is impregnated with melted cacao-butter, and embedded in a cake of the same material. There is hardly any limit to the thinness with which sections from a piece so embedded may be obtained, and all that is further necessary is to dissolve away the included cacao-butter from the sections with oil of cloves and to mount them in dammar.

Preparation 2. Fat absorption.—For the purpose of studying the course which fatty particles take in passing from the cavity of the intestine into the central lacteals of the villi, an animal is killed three or four hours after a meal composed almost exclusively of fat (it should previously have been allowed to fast for several hours). On opening the abdomen the lacteals in the mesentery will be found filled with chyle, and the cavity of the small intestine occupied by emulsified fat which is undergoing absorption. The intestine is opened at once, and two or three very small pieces of the mucous membrane are snipped off and placed in 1 per cent. osmic acid solution. Another minute piece is placed in a drop of serum or aqueous humour and is quickly teased-out with needles; a piece of hair is added, and the preparation is covered and examined. One of the portions in osmic acid is allowed to remain forty-eight hours in the solution, and is then broken up in water. The others are transferred to dilute Kleinenberg's solution, and when stained throughout are embedded by the cacao-butter process. The sections are placed, after the cacao-butter has been extracted from them by warm oil of cloves, first in spirit, and then in water and are finally mounted in glycerine.

In the two teased preparations—serum and osmic—many of the columnar epithelium cells will be found to contain fatty globules of various sizes (stained black in the osmic preparation). Similar, but for the most part smaller particles will also be found in the numerous lymphoid corpuscles which are set free from the retiform tissue of the mucous membrane by the process of teasing. In the sections the epithelium cells and the lymph corpuscles will be observed, *in situ*, in the same condition, viz. containing blackened fatty particles, and moreover the cleft-like central lacteal in the middle of each villus will be found to contain similar globules. Hence we infer that the fatty matters are first taken up from the cavity of the intestine by the columnar epithelium cells; that they are transmitted in some way from these to the amœboid lymph cells, and that these again convey them to and discharge them into the central lacteal.

Preparation 3. Vessels of the small intestine.—The *bloodvessels* of the small intestine are to be studied by aid of vertical sections of the injected gut. The sections may be lightly stained with logwood.

The *lymphatics* (lacteals) may perhaps be seen in thin sections of the uninjected preparations as cleft-like spaces in the villi and in the substance of the mucous membrane, and surrounding the bases of the lymphoid nodules which make up the Peyerian patches. It is not an easy matter to inject those of the mucous membrane, although the larger plexuses of the submucous and muscular coat can be more easily demonstrated.

Preparation 4. Nerves of the intestinal wall.—The nerves of the intestinal canal form a very interesting subject of study, comprising two of the closest and most richly gangliated plexuses of pale fibres which are met with in the animal body. They may moreover, by following the method here to be described, be shown without any great difficulty in all parts of either the small or the large intestine. It is preferable to choose an animal (*e.g.* rabbit or guinea-pig) in which the intestinal coats are not very thick. The following is the mode of procedure: A piece of glass tubing about a quarter of an inch in diameter and five or six

inches long is taken and one end is drawn out into a cannula, whilst to the other a small piece of indiarubber tube, furnished with a spring clip, is attached. Chloride of gold solution ($\frac{1}{2}$ per cent.) is drawn up into the tube so as almost to fill it, and the clip is then closed, to prevent the escape of the fluid. Care should be taken not to suck any of the gold solution into the mouth. A piece of intestine about three inches long is removed from the dead animal, and if not already empty its contents are washed out by a stream of salt solution. The intestine thus emptied and cleaned is ligatured firmly at one end, whilst into the other is tied the cannulated end of the glass tube containing the gold solution. When thus secured the clip is opened and the fluid is allowed to flow into and distend with moderate force the piece of gut, the action of gravity being assisted by gently blowing through the indiarubber tube. As soon as the intestine is filled with the gold solution the clip is again allowed to close, and then, while an assistant holds the glass tube in a vertical position, the operator ligatures the gut just beyond the end of the cannula, which may now be cut away. The piece of intestine, thus filled with the gold solution, is immersed for an hour in more of the same liquid. It is then placed in a dish of water and cut open longitudinally with scissors, so as to allow the contained fluid to escape, after which the puckered, ligatured ends may also be removed. The tissue being hardened by the gold solution, the piece of gut which remains retains its cylindrical shape. It is well to halve it by another longitudinal cut, so that both inner and outer surfaces may be freely exposed to the light. The pieces are now placed, with their outer surfaces uppermost, in a glass vessel of water containing just enough acetic acid to be sour to the taste, and the vessel is covered and allowed to stand in a warm place freely exposed to the sunlight (see p. 79). After two days its colour will be found to have changed to a dark violet. A few drops of methylated spirit may then be added to the fluid; this serves to aid the reduction of the

gold and to prevent the growth of fungi. In another day or two the tissue will be so dark as to appear almost black. A portion is then removed to a glass dish of water and prepared in the following way. In the first place, the glandular mucous membrane is separated from the rest of the intestinal wall either by tearing it off with forceps or by scraping it away with the end of a blunt scalpel. There now remain the serous and two muscular layers, together with the submucosa. To the inner surface of the latter the muscularis mucosæ may be still adherent. The separated fragments of the mucous membrane are got rid of by pouring away the water first used and substituting fresh, and then an attempt must be made, by aid of two pairs of forceps, to peel the submucosa off from the inner surface of the muscular coat. Of course if the muscularis mucosæ has been left, that will form a part of the layer which is thus removed. The separation must be done slowly and carefully, so as to get as large a piece as possible intact. When this is accomplished satisfactorily a slide is immersed in the water, and the portion of submucosa so detached is floated on to it, and removed from the water. Its further preparation consists in allowing the excess of water to run off, applying a coverglass, making sure first of all that the layer is free from folds, and then allowing glycerine to pass under the cover-glass and replace the water as this evaporates.

Returning to the remainder of the piece of intestine, the next process consists in picking away bit by bit with forceps the comparatively thick layer of circular muscular fibres. This is not a difficult proceeding, and when it is finished all that remains is the thin serous coat and the longitudinal muscular layer, to the inner side of which the nervous plexus of Auerbach, the intermuscular plexus, is adherent. No further separation is required, all that is necessary being to float the piece of tissue on to a slide with the (concave) inner surface uppermost. But before applying the cover-glass the preparation is to be examined

with a low power, to see that the surface of the serous membrane is free from a finely granular precipitate which is apt to be deposited in the acidulated water. If this is present, the piece must be replaced in the water and the precipitate gently brushed off with a soft camel-hair pencil. The preparation is completed in the same way as that of the submucosa. The latter shows Meissner's plexus, the cords of which are much finer than those of Auerbach's. In both plexuses the nervous cords are stained of a violet colour by the reduction of the gold; at the points of junction of the nervous cords are groups of small ganglion cells, the nuclei of which are hardly stained at all, and consequently look clear in the midst of the darkly-stained cell-bodies. The distinction between the individual cells is difficult to make out. Branches may perhaps be traced passing from the plexus of Auerbach amongst the muscular fibre-cells: from that of Meissner to the muscularis mucosæ, if this is present, and perhaps also to the small bloodvessels, which are particularly well seen in the preparation of the submucous coat.

Preparations 5–7. Large intestine.—For hardening the tissue and preparing sections of the large intestine the same methods are employed as for the small intestine, so that it is unnecessary to recapitulate them.

The injected large intestine is prepared, like the stomach, by means of vertical and horizontal sections.

The lymphatics are not easy to inject, but present less difficulty than those of the stomach.

CHAPTER XVI.

THE LIVER.

Preparation 1. Uninjected liver.—To prepare sections of the liver small pieces are placed in 2 per cent. bichromate of potash solution for ten days, transferred from this to weak spirit, and in twenty-four hours are placed in strong spirit, to complete the process of hardening. The tissue will be hard enough to cut thin sections from in another day or two. The sections are stained with logwood, and mounted in dammar varnish. They should be made in two directions, viz. (1) in a plane near and parallel to one of the surfaces of the liver, and (2) vertical to the surface. Those made in the direction first named will for the most part cut the central or intralobular veins across, those in the second direction may take them along their length ; the apparent arrangement of the blood capillaries and liver cells in the individual lobules will differ, both in accordance with this difference of direction and also according as the lobule is cut exactly through its centre or at some part more or less removed from this. Between the lobules are seen the branches of the portal vein, always accompanied by a branch of the bile duct, the columnar epithelium of which is very well seen in these preparations, and by a branch of the hepatic artery. All three are included in a mass of connective tissue, a prolongation of Glisson's capsule, enclosing them in a so-called portal canal. In this connective tissue cleft-like spaces may generally be seen—two or three in the section of a portal canal—not merely

breaks in the connective tissue, but with quite a definite wall. These are the accompanying lymphatics. Other lymphatics accompany the branches of the hepatic veins, but are not so easily seen in the sections, although they can be injected. The branches of the hepatic veins are readily distinguished from those of the portal vein, by the fact that they run unaccompanied by branches of the bile duct and hepatic artery. The blood capillaries of the lobules look like spaces (filled with round clear bodies, the altered blood corpuscles) between the rows of cells (in the sections these appear arranged simply in rows) ; their walls are very thin, and the hepatic cells appear for the most part to come in contact with the wall. But in reality there is a second delicate membrane around many of the capillaries, and between it and the epithelioid wall of the vessel is a space for the passage of lymph (perivascular lymphatic) ; it is difficult to make this out, however, in preparations in which the lymphatics are not injected. The round nuclei of the liver cells are deeply stained by the logwood, and the cells themselves slightly. In the thinnest parts of the sections the lines of junction between neighbouring cells can be well made out, and not unfrequently the small capillary passage for the bile which intervenes between the adjacent sides of the cells can, according to the direction in which it runs, be recognised with a very high power either as a horizontal line or as a minute aperture. To obtain the best results the pieces of liver, which are not to be more than an inch or so square and a quarter of an inch thick, should be placed in the bichromate solution quite fresh, from an animal killed only a short time previously.

Preparation 2. Injected liver.—The vessels of the liver seldom get filled when the rest of the body is injected from the aorta. It is generally necessary to make a special injection of this organ from the portal vein. For this purpose the usual red or blue gelatine injection is used, the apparatus being arranged as described at p. 146. The operation is conducted as follows :—The animal (rabbit) having been killed

by bleeding,[1] the thorax is opened; and the pericardium being torn away, the heart is raised and two thread ligatures are passed round the inferior vena cava. One of these is tightened as near the heart as possible, and then a snip is made in the vein, so as to allow the blood to escape freely. Next, the abdomen is opened, and the intestines and stomach being gently drawn to the left side, the peritoneum at the back of the abdomen is torn through, and a ligature placed around the vena cava above the accession of the renal veins. The portal vein is then found in the fold of peritoneum which connects the under surface of the liver with the stomach, and a ligature, in the noose of which the hepatic artery may be included, having been passed round it near the liver, a snip is made in the vessel, and the injecting cannula is tied in. This cannula is now filled by means of a pipette with warm salt solution, and the supply tube (from the injecting bottle) having been completely filled by the injecting fluid to the exclusion of air in the same way as in the first injection (p. 147), is slipped over the open end, and the injection at once allowed to flow. As it passes by the portal system of veins through the lobules of the liver into the hepatic system, it forces whatever blood is still contained in the bloodvessels of the organ out into the vena cava, whence it can freely escape into the thorax through the snip which was there made in the vein. As soon as all the blood is thus driven out, and only pure injecting fluid begins to pass, this vein is occluded near the diaphragm by the second thread.

[1] In injecting the whole body it was recommended to kill the animal by chloroform. This was for the purpose of having the bloodvessels as much dilated as possible. When an animal is killed by bleeding the arteries contract very considerably, and, remaining contracted some little time after death, offer a considerable resistance at first to the passage of the injection, and this may tend to spoil the result altogether. In the liver, however, the case is different, since it is not injected through arteries, but through veins, which possess little contractility. Any blood which remains in the vessels does not, so long as it remains fluid, impede the passage of the injection, but it is driven before it.

The pressure in the injecting bottle is then slowly raised, but should not even at the utmost exceed three inches of mercury, for this amount of pressure will cause all the bloodvessels to be quite fully distended, and will effect a very considerable consequent enlargement of the organ; more might cause rupture and extravasation. After the lapse of a few minutes, to allow of the complete filling of all the blood-vessels, a second ligature is tied round the *portal* vein close to the liver to prevent the return of the still fluid injection, and the cannula is cut out from the portal vein (the pressure in the apparatus having first been removed), and the body put into a cold place so as to permit the gelatine to solidify. It is well to hasten the process by pouring cold water—iced if possible—over the liver. When the injecting material is entirely set, the organ is removed and cut into pieces, which are placed in weak spirit (half water) for twenty-four hours. They are then put into stronger spirit, and in forty-eight hours more in the strongest. In three or four days sections may be made (in two directions as with the uninjected organ), and mounted, after passing through oil of cloves, in dammar. It is better not to stain them.

During the whole process, the greatest care must be taken not to handle the liver more than can possibly be helped, for it is very readily scratched or ruptured, and any such accident would tend to permit the escape of the fluid injection. This warning applies with equal, if not greater, force to the operation next to be described; that, namely, of filling the bile-ducts.

Preparation 3.—The bile-ducts are injected with Berlin blue solution, 2 per cent., the mercury apparatus (fig. 30) being used. The solution, although fluid in the cold, should nevertheless be employed warm, as it is then less likely to excite contraction of the biliary ducts. A rabbit is killed by bleeding, the abdomen opened, and the common bile-duct sought for close to the portal vein; a ligature is passed round it and a small piece of card being placed under as a support,

and to separate it from the accompanying vessels, a snip is made into it, and a glass cannula is inserted, and having been passed along the duct as near to the liver as possible is tied in. The cystic duct is ligatured to prevent the injection from passing into the gall bladder. In the next place the cannula is filled with warm Berlin blue solution by means of a fine pipette; the (previously filled) supply tube is attached, the clip on this opened, and the pressure gradually raised to about two inches of mercury. The blue fluid, driving whatever bile there happens to be left in the ducts before it into the lobules, penetrates first into the interlobular bile-ducts, and from these into the outer parts of the lobules, forcing the bile more and more towards the centre; here of course there is no escape for it, except that a little may pass into the lymphatics and blood-vessels through their walls. Hence it will be understood that the injection can only be made to fill the intercellular biliary passages in the *outer* part of each lobule. The injection should be persevered with for about half an hour; the bile-duct may then be tied and the injecting apparatus removed; after which the liver is cut out entire, without injuring it in any way, and placed in strong spirit. In twenty-four hours it is cut in pieces, and the spirit changed, and in less than a week the pieces will be hard enough to cut. The sections may be stained slightly with logwood.

Preparation 4. Lymphatics of the liver.—The lymphatics of the liver are injected through a fine cannula stuck obliquely into the superficial part of the organ immediately beneath the capsule. Either solution of Berlin blue or alkanet may be used. The part should be quite fresh. If the injection be persisted in for a long while the fluid may flow out both by the lymphatics accompanying the portal vein and those accompanying the hepatic veins (Ludwig and Fleischl). Very frequently, however, the injecting fluid finds its way into the blood-system instead of the lymphatics. The injection of the lymphatics may be accomplished in another manner, viz. by seeking those lymphatics which accompany the hepatic veins at the back of the liver, and tying a cannula into them. After a time the fluid will be found to pass out by the vessels which accompany the portal vein.

The investigation of the lymphatics of the liver is, however, very difficult, and our knowledge of their course and arrangement is still by no means satisfactory.

Preparation 5. Hepatic cells.—In addition to what may be learnt from sections of the organ, teased out preparations afford much useful information, both of the characters of the liver cells and of the connective tissue of the lobules. For this purpose small portions of the perfectly fresh and warm liver are broken up in serum or salt solution, and other portions are macerated for a day or two in weak bichromate of potash, and subsequently teased out in water.

The **pancreas** is prepared in the same manner as the salivary glands, to the description of which the student is referred.

CHAPTER XVII.

THE SPLEEN AND URINARY ORGANS.

THE SPLEEN.

Preparation 1. The uninjected spleen.—The spleen is hardened in the same manner as the liver, by placing small pieces of it in 2 per cent. solution of bichromate of potash, and in about a week or ten days transferring them first to weak and then to strong spirit. The sections, which cannot be too thin, are to be stained deeply with logwood, and mounted by the ordinary mode of procedure in dammar varnish.

In these preparations the Malpighian corpuscles (or nodules of lymphoid tissue) are very strongly coloured, as are also the trabeculæ which traverse the pulp, especially in those animals in which they are largely composed of plain muscular tissue; the substance of the pulp is but slightly coloured by the logwood, only the cell-nuclei, and, to a much less extent, the branching cells of the retiform tissue being stained. The prevailing colour of the pulp is yellowish, owing to the blood (altered in colour by the action of the reagent) which at the time of death remained in the interstices of the tissue. Moreover, here and there a speck of coarsely-granular reddish yellow pigment may be detected, lodged in one of the corpuscles of the spleen pulp. But this will be better made out in the teased-out preparations subsequently to be described.

Preparation 2. Irrigated spleen.—By another mode of preparing the spleen all the blood is first washed out by a stream of

salt solution, injected through the splenic artery, and the organ is then hardened in the same way as before. It will much facilitate the process of hardening if the salt solution is followed by a stream of the bichromate. This may even be made to distend the organ somewhat, the distension being maintained by ligaturing the vessels near the hilus. In such a case the organ is to be placed entire in 2 per cent. bichromate, and only cut into pieces after forty-eight hours. For a spleen which has been thus prepared, the cacao-butter method of embedding may be employed, after a thin piece has been stained by alcoholic logwood. By thus removing the blood corpuscles the retiform tissue of the pulp is better seen. Klein (Quarterly Journal of Microscopical Science, October, 1875), relying on appearances presented by preparations made in this way, describes the retiform tissue of the spleen as entirely made up of flattened cells forming by their junction a 'honeycomb of membranes.' That such a description is far too exclusive, the study of teased preparations amply demonstrates, for the *network of branched cells*, described by almost all previous observers, is readily seen in them (see below, Prep. 4).

Preparation 3. Injected spleen.—The spleen may have been injected in the animal which was injected entire; if this is not the case, a special injection is to be made from the splenic artery. When successfully accomplished, the vessels are as usual ligatured to prevent the escape of the injection, and the organ is immersed entire in spirit, at first weak but with the strength afterwards gradually increased, as in the case of the liver. The sections will show what at first sight look like accidental extravasations, large patches namely of injection distributed all over the organ, with the exception of large round white patches here and there, pervaded by a few capillaries. The white patches are sections of the Malpighian corpuscles, and the part permeated by the injection is of course the pulp, into which the arterial capillaries freely open.

Preparation 4. Splenic cells.—To obtain specimens of the spleen substance, which will show in a separated condition the cellular elements which it contains, and of which it is composed, a small portion of the fresh organ may be teased

out with needles in a little salt solution or serum. But it will be found that so much blood is incorporated with the spleen substance (it forms, in fact, most of the soft matter which can be expressed from the fresh section) that the view of the other parts is obscured by innumerable red blood corpuscles. Hence before teasing a piece it should be placed for forty-eight hours in $\frac{1}{8}$ per cent. bichromate of potash solution. This destroys the red corpuscles whilst preserving the character of, and at the same time macerating somewhat the proper substance of the spleen, so that the cells are now readily separated and seen. By far the greater number are lymph corpuscles from the lymphoid tissue of the Malpighian nodules and of the arterial adventitia. But, besides these, other cells are met with; larger, often flattened, and many of them with fine branchings. These are cells of the retiform tissue; some of them contain pigment granules as already intimated. They may be found either entirely isolated, or forming a fine network by the intercommunication of their branches. Their nuclei, as well as those of the lymphoid cells, are well brought out if a little logwood solution is permitted to pass under the coverglass. In the fresh preparations, not treated by bichromate of potash or any other reagent, but made in serum, some of the cells may perhaps be found containing red blood corpuscles in their interior, and transitions from these to those containing pigment are met with.

THE KIDNEY.

Preparation 5. The uninjected kidney.—The kidney is hardened in the same way as the liver and spleen, viz. by a strong solution of bichromate of potash (2 per cent.). The piece or pieces that are employed should include both cortical and medullary parts; but at the same time should not be thicker than from a quarter of an inch to half an inch, otherwise the preservative fluid will not penetrate rapidly enough

to the deeper parts. They are to remain in the bichromate solution (a relatively large quantity) for three weeks; are then placed in weak spirit, and in twenty-four hours transferred to strong spirit. In three or four days more the pieces are firm enough to cut. They will perhaps be large enough to hold in the hand, and thus the necessity of embedding will be avoided; sections as thin as possible are to be made in a plane vertical to the surface of the organ, and large enough to include both cortical substance and Malpighian pyramid. One such section is first transferred from the spirit to water, and is then simply mounted in glycerine; another is stained with logwood, and after treatment by the customary process is mounted in dammar varnish. These sections will show; in the cortical substance the Malpighian corpuscles, the convoluted tubules variously cut, and the prolongations of the straight tubules of the medullary substance and of the tubules of Henle; in the pyramidal part the two last-named tubules, and the collecting and excretory tubules, seen longitudinally, with a large number of bloodvessels running parallel to and between them.

Preparation 6.—A transverse section also should be obtained of the medullary substance. With this object the end of a pyramid is cut off with the razor, and, as it is too small to hold in the hand, it is embedded in wax-mass in such a position that the tubules will be cut exactly across. Sections from this are mounted like the others in glycerine and in dammar varnish.

The kidney of the dog or of almost any other animal may be used for the above preparations, and has the advantage over the human organ, that it is obtainable in a fresher condition. But since the epithelium of the tubules, at all events of the convoluted tubules, differs somewhat in appearance in the human kidney as compared with that of the lower animals, a portion of a perfectly-healthy organ should be procured from the post-mortem room as fresh as possible, and prepared for observation in the same manner.

Preparation 7. The injected kidney.—The bloodvessels of the kidney will very probably be filled in injecting an animal entire; but, if this should not have been the case, it is not difficult to make a special injection of the separated organ from the renal artery. The red gelatine injection may be used, and the kidney is kept warm, and the injection maintained for a considerable time, in order that the vessels of the glomeruli and the network of capillaries in the cortical substance supplied by their efferent vessels may be completely filled. The organ is then set aside in a cool place (surrounded by ice, if possible), and, when the gelatine is completely set, is cut into three or four pieces and hardened gradually, as usual, with alcohol. The sections, which need not be very thin, but should be quite even, and comprise the whole thickness of the organ, are to be mounted, unstained, in dammar varnish.

Preparation 8. Uriniferous tubules.—The uriniferous tubules may be injected from the ureters for a considerable part of their length, simultaneously, if it be desired, with the injection of the bloodvessels by a solution of Berlin blue. But even if well filled they are too densely arranged to render it possible to trace individual tubules along their whole extent. This may be better accomplished by making teased preparations of the kidneys of small animals, which have undergone some process of preparation, having for its object the solution or softening of the intertubular substance. Several such processes have been proposed, but none yield entirely satisfactory results. The best, perhaps, that has yet been tried consists in digesting tolerably thick slices of a small kidney in a mixture of 4 parts of spirit and one of hydrochloric acid, kept boiling for 3 or 4 hours. The boiling is performed in a flask fitted with a cork, through which a long vertical tube passes; in this much of the vapour which is driven off by the boiling becomes condensed, and flows down again into the flask. After the time mentioned the slices are removed and placed in water; and, after lying in this for a few days, minute shreds, comprising the whole depth from external surface to papillæ, are split off with needles, placed on a slide, and unravelled as much as possible by aid of the dissecting microscope. The preparation is covered with a specially large piece of covering-glass (a hair being first added to avert

the pressure of the glass on the soft tubules), and stained by drawing picric acid solution under the coverglass. This soon colours the tissue intensely yellow; glycerine may then be allowed to pass in at the border in order to complete the preparation. Some of the tubules will be found isolated for a considerable part of their length, and the passage of the convoluted tubules into the looped tubes of Henle may especially be well seen. The epithelium of the tubules is for the most part well preserved, but that of the convoluted ones has become very granular, and so swollen out as to completely fill up the tubule.

Preparation 9. Examination of the fresh kidney.—When by these various means sufficient acquaintance has been gained with the various tubules and their contents *in situ*, the examination of the fresh tissue in serum may be attempted. With this object small snips are to be made from different parts of a freshly-cut surface with a pair of curved scissors, and teased out in a drop of serum, with the aid of the dissecting microscope, so as to separate as many of the tubules as possible. In doing this much of the epithelium will become detached, and the characters of the individual cells in the fresh condition may be studied.

Preparation 10.—The epithelioid cells of the capsules of Bowman and of the basement membranes of the tubules may be shown by nitrate of silver. For this purpose a fresh kidney is sliced in half by a single cut with a sharp razor in the direction of the tubules. One of the halves is thoroughly washed with distilled water, and solution of nitrate of silver ($\frac{1}{2}$ per cent.) is poured over the cut surface. After a minute and a half the silver solution is rinsed off with distilled water, and the piece of kidney is placed in a beaker of strong spirit, with the silvered surface exposed to the sunlight. When brown it may be removed from the light, but is left in the spirit for twenty-four hours; one or two sections are then made from the brown surface, clarified in oil of cloves, and mounted in dammar.

THE SUPRARENAL CAPSULES.

Preparation 11.—To prepare the suprarenal capsule it is separated from the surrounding fat, divided into two or

three pieces by transverse cuts, and placed in 2 per cent. bichromate of potash solution for fourteen days, when the hardening may be completed in spirit in the usual manner. Hardening the organ in spirit alone also gives very good results. The mode of preparing the sections, which should include both cortical and medullary substance, calls for no special description.

If the human suprarenal is not obtainable in a fresh condition (the medullary substance very readily softens and breaks down after death), that of the guinea-pig, which is large comparatively to the size of the animal, and has the distinction between cortical and medullary substance well marked, may advantageously be employed.

Preparation 12.—In a teased-out preparation of the fresh organ the cellular elements of the cortical and medullary substance may respectively be studied, and the effect of a solution of yellow chromate of potash in colouring the medullary cells brown may be observed.

THE URETERS.

Preparation 13.—The ureters are prepared in the same way as the intestine—by moderately distending an excised portion with a mixture of equal parts of spirit and $\frac{1}{2}$ per cent. chromic acid solution, and placing the piece thus distended in a beaker containing some of the same mixture. After twenty-four hours the tube is slit open, and transferred to spirit for two or three days. The sections are to be made across the length of the tube, and stained and mounted in the ordinary manner.

Preparation 14. Epithelium of ureter.—To study the separated epithelial cells a piece as fresh as possible is cut open, pinned out on a cork with the inner surface uppermost, and immersed in $\frac{1}{8}$ per cent. bichromate of potash solution for from twenty-four to forty-eight hours. Some of the epithelium is then scraped off with a spear-shaped

needle or the end of a scalpel, and is broken up in a drop of water. After the addition of a piece of hair to the fluid the coverglass may be applied, and the preparation examined with a high power. If it prove successful, with many of the epithelial cells fully separated, it may be permanently preserved. With this object the cells should first be stained, by allowing weak logwood solution to run under the edge of the coverglass. The logwood is to be followed by a drop of glycerine applied at the same edge; and, when the glycerine has become diffused underneath, all that is necessary is to cement the coverglass.

THE BLADDER.

The urinary bladder, both for sections and teased-out preparations, is prepared by exactly the same methods as the ureters. To distend it a glass cannula, connected by an indiarubber tube with a bottle containing the chromic fluid, is tied into the urethra. The organ must not be over-distended, but only moderately filled. Any urine which it may contain should first be allowed to run out through the cannula.

CHAPTER XVIII.

THE GENERATIVE ORGANS.

Preparation 1.—Those parts which contain erectile tissue will be best studied after having been injected. Their blood-vessels and sinuses may have been filled in the animal which was injected entire from the root of the aorta; but, if not, a special injection from the lower end of the abdominal aorta is to be made, the arteries supplying the lower limbs being first tied to prevent waste of the injection. The hardening of the parts in spirit must be effected very gradually (the spirit being daily made stronger), since otherwise the gelatine shrinks away from the walls of the venous sinuses, and the preparation becomes in great measure spoiled. The sections which are made should some of them be mounted, unstained, in dammar, others after being lightly stained with logwood, so that the plain muscular and fibrous tissue, and also, in sections including the urethra, the epithelium of that tube may be exhibited as well as the vessels.

Preparation 2.—Parts which have not been injected are hardened in 2 per cent. bichromate of potash solution (fourteen days), or $\frac{1}{6}$ per cent. chromic acid (seven days), followed by spirit in either case.

Preparation 3.—The glandular organs, such as the prostate and vesiculæ seminales, are prepared either with the $\frac{1}{6}$ per cent. chromic acid solution followed by spirit, or with spirit alone. The subsequent processes of staining and mounting are the same for all, except that it will be found, as a

rule, that those which have been in chromic acid stain less readily than those which have simply been hardened in spirit.

Preparations 4–6.—The scrotum, and labia, and the vagina, are prepared in the same way as the skin (see p. 166).

Preparation 7.—The human uterus is best hardened in the 2 per cent. bichromate of potash; its cavity should be freely laid open. In animals (the rabbit, for instance), where it is more membranous, the uterus and the upper part of the vagina may be prepared together by distending them with the spirit and $\frac{1}{2}$ per cent. chromic acid mixture through a cannula tied into the lower part of the vagina. The vagina is then tied, and the organs are cut out and placed in a quantity of the solution; in twenty-four hours they are laid open and the fluid renewed, and in another day or two are ready to be put into spirit. The sections are stained with logwood, and mounted in dammar.

Preparation 8. Section of ovary.—The ovaries are prepared by placing them—with as little handling as possible, so as to avoid rubbing off the columnar epithelium which covers the surface—in $\frac{1}{6}$ per cent. chromic acid (whole if taken from a small animal, such as a rabbit or cat; cut into two or three pieces if from a larger one). In most of the lower animals they must be sought much higher in the abdomen than in the human female; in the rabbit they occur as small elongated bodies, dotted all over with little projections (the Graafian follicles), and situated just below the kidneys. They are left in the chromic solution for seven days, and then placed in spirit, and in two or three days more are ready for cutting. The hardening is effected still more readily by using the chromic and spirit mixture. The sections are to be stained in carmine solution; for logwood sometimes colours very deeply the coagulated fluid in the Graafian follicles, so that the epithelial contents are obscured: this colouration is probably owing to the presence of mucus (or rather mucin) in the secretion of the follicle. After having been stained the sections are passed through alcohol and oil of cloves, and mounted in dammar.

Preparation 9. The ovum.—The ripe mammalian ovum, although it can be seen within the larger Graafian follicles in the sections of the hardened organ, forms a much more beautiful object when obtained isolated from the ovary of a recently-killed animal. A full-grown doe rabbit, not pregnant, is to be sacrificed for this purpose. One of the ovaries having been removed, it is held firmly between the finger and thumb over a clean glass slide, in such a position that the largest and most prominent Graafian follicle is almost in contact with the middle of the slide. The follicle is then pricked with a sharp-pointed scalpel, so as to cause the fluid contents of the follicle to spirt out, carrying with them the ovum, surrounded by a number of the epithelium cells. The ovum is rather too small to be detected with the naked eye, but its presence may be suspected if, on glancing at the slide in such a manner that the light is reflected from the surface of the fluid to the eye, a slight prominence is observed on the otherwise flat surface. Its presence here is confirmed by examination with a low power, and it may then be carefully observed with the ordinary high power. It is better, if possible, not to apply a coverglass, for the zona pellucida is apt to become broken ; and, moreover, even slight pressure spoils in great measure the natural appearance of the object. But if the objective becomes dimmed by its proximity to the fluid, or if it is desired to employ an immersion, then a thin coverglass must be used, and to protect the ovum from pressure a narrow slip of thick paper (an ordinary hair is not thick enough) is to be put on either side before the coverglass is applied. If the fluid which accompanies the ovum from the Graafian follicle is not in sufficient quantity, a drop of aqueous humour may be added to it. It is not possible to preserve this preparation permanently.

Preparation 10. Artificial impregnation.—If a preparation similar to that just described is examined on the warm stage at the temperature of the body, and a little of the seminal secretion of a male rabbit with the spermatozoa in

full activity is added, the penetration of these into the zona pellucida of the ovum can be observed.

Preparation 11. Section of uninjected testis.—The testis is one of the most difficult organs to prepare, owing to the looseness of its structure. It is firmest in those animals (cat, pig) in which the peculiar, granular, polyhedral cells, which accompany and surround the bloodvessels, are most numerous. To harden it two or three cuts are made almost through its whole thickness, and it is then placed for ten days in 2 per cent. bichromate of potash solution, and afterwards in spirit. Sections of both the body of the testis and the epididymis are to be made.

Preparation 12. Lymphatics of Testis.—In the sections made as above there will be observed in the interstices between the seminiferous tubules large, cleft-like spaces, looking almost like accidental clefts in the loose connective tissue uniting the tubules. They are in reality, however, the lacunar commencements of the lymphatics. To show this the following simple experiment may be performed:—In a recently-killed dog the fine cannula of a Pravaz syringe, filled with Berlin blue solution, is stuck through the scrotum into the middle of the substance of one of the testes, and the solution is slowly, and without exerting any considerable pressure, injected into the organ. If the abdomen is opened the blue fluid will soon be seen passing along the lymphatics which run in the spermatic cord, and from these into those of the back of the pelvis and abdomen, at length reaching the thoracic duct. If now the testis is removed and hardened in spirit, and sections are made of the hardened organ, it will be found that the intertubular spaces are occupied by the blue substance, and, since they are proved by the injection to be in free communication with the lymphatics which leave the organ, the spaces are to be looked upon as giving origin to the lymphatics.

Preparation 13. Isolation of the seminiferous tubules.—For obtaining the tubules isolated for a considerable length, pieces of the testis (preferably human) are placed for a day or two in hydrochloric acid, diluted with $\frac{1}{3}$ its volume of

water, and maintained at 30° C. They are then allowed to lie in water until the tubules can be readily separated with needles (Mihalkovics, Ludwig's 'Arbeiten,' 1874). Teased-out preparations of the fresh testis-substance are also to be made in serum, to exhibit the form, stages of development, and movements of the spermatozoa. For the object last named the preparation should be examined on the warm stage.

Preparation 14. Epithelioid cells of seminiferous tubules.—To exhibit the fact that the apparently structureless basement membrane of the seminiferous tubules is in reality composed of layers of flattened epithelioid cells, a portion of the testicular substance is partially unravelled in distilled water, and some of the tubules which are thus isolated are dipped into nitrate of silver solution for a minute, and, after being again rinsed in water, are mounted in glycerine and exposed to the light; the lines of junction between the flattened cells are by this means made evident.

Preparations 15, 16. Tunica Vaginalis.—The tunica vaginalis is to be prepared in the same way as the other serous membranes (with nitrate of silver), partly unbrushed to show the epithelioid covering, and partly brushed for the sake of exhibiting the parts beneath. For the preparation of the visceral part, the process is similar to that adopted for the pericardium covering the surface of the heart (p. 171), and need not here be more particularly described.

Preparation 17. The Mammary Glands.—Small pieces of these organs are hardened by being placed in $\frac{1}{6}$ per cent. chromic acid solution for a week or ten days, subsequently transferring them to spirit. The mixture of equal parts of spirit and $\frac{1}{2}$ per cent. chromic acid may also be used and produces the desired result more rapidly (in two or three days); moreover the sections stain more readily with logwood. They may be mounted either in dammar or in glycerine. The appearances presented by the cells of the alveoli vary considerably according to the state of functional activity of the gland. (See Creighton, Report of the Medical Officer to the Privy Council, 1875, and Journal of Anatomy and Physiology, October, 1876.)

CHAPTER XIX.

THE CENTRAL NERVOUS SYSTEM; THE BRAIN AND SPINAL CORD.

SOME experience has already been obtained of the methods which are employed for studying the cellular elements of the central nervous system in an isolated condition (see p. 112). The spinal cord was the part then under investigation, but the nerve-cells found in the grey matter of the cerebrum and of the cerebellum may be observed in the same manner with equally satisfactory results. Without delaying, then, to repeat the directions there given, we may pass on to describe the best methods of preparing *sections* of the parts in question.

Preparation with bichromate of ammonia.—To harden any part of the central nervous system the most generally useful reagent is the bichromate of ammonia (2 or 3 per cent. solution). This will of itself render the tissue sufficiently firm for obtaining thin sections, but it is always best to finish first with weak and then with strong spirit, especially as this fluid must be used to wet the razor. The pieces to be hardened should not be too large, or at all events not too thick, but the solution has considerable power of penetration (differing in this respect from chromic acid), and the whole length of the spinal cord of any of the smaller quadrupeds, and even that of man, may be hardened in it intact if put in perfectly fresh. It is always better, however, to cut it into short lengths. The pieces are ready to be transferred from the bichromate of ammonia solution to dilute spirit in three or four days if small;

in a week if rather larger: after twenty-four hours they are placed in strong spirit.

In this way a piece of the cerebellum, two or three small pieces from different parts of the convoluted surface of the cerebrum, the medulla oblongata, and pieces of the spinal cord from the middle of the three regions (cervical, dorsal, and lumbar) are to be prepared. They should be taken from the human subject if possible, although it is useful to study the parts, especially the spinal cord and medulla, in the lower animals as well. The sections, at least those of the spinal cord and medulla, may be made with the aid of a microtome (see Appendix), and, after being stained, are treated in the usual manner with alcohol and oil of cloves, and mounted in dammar varnish. Logwood may be used for the purpose of staining the sections, and also carmine; but, on the whole, for sections of the central nervous system, some of the aniline dyes (which may for the most part be employed in either alcoholic or watery solution) give better results. One of the best is that known in commerce as aniline-blue-black, which stains the nerve-cells and the axis-cylinders of a dark slate-blue colour. For the cerebellum a solution of aniline blue is recommended.

A double colouration by logwood and eosine (an aniline dye, possessing a red colour) has been recently advocated. The eosine is to be used in solution in alcohol (see Appendix). The sections are first stained with logwood, and then placed in eosine. When sufficiently coloured by this they are passed through oil of cloves and mounted in dammar.

Preparation by Sankey's method.—For the preparation of large sections of different parts of the brain (particularly of the cerebral convolutions and of the cerebellum), destined more particularly to show the nerve-cells and the course and connection of their offsets, there is no better method than that described by Sankey ('Quarterly Journal of Microscopical Science,' April 1876). It is shortly as follows:—With a large and long knife wetted with spirit, slices of the fresh brain (of the cerebellum across the laminæ, for example)

are made, as thin as possible under the circumstances—one-tenth of an inch thick perhaps—and are placed in a strong watery solution (7 per cent.) of the aniline-blue-black. After three hours the staining solution is poured off and water substituted, and in a few minutes this, which will still be intensely coloured, is replaced by fresh water, and so on until the excess of the staining fluid is washed away from the slices. If one is cut in half it will be seen that only the surfaces are stained, the aniline solution not having penetrated to the interior. When the washing is completed the slices are transferred from the water to glass slides. This can be effected, without risk of breaking the sections, by floating them on to an immersed slide, and raising both together out of the water. The excess of water is then allowed to drain off, and the pieces are left in a dry place exposed to the air, so that after twenty-four or at most forty-eight hours, they will be found firmly dried to the glass. In the process of drying they will have lost a good deal in thickness, and this is now still further reduced by planing off the upper stained surface with a razor, or other suitable instrument, without scraping away at any place, if it can be helped, the lower stained part which is adherent to the slide. The further preparation consists in covering the section with a thin layer of dammar varnish (oil of cloves is not requisite) ; after which it may be examined with the microscope, and, if it appear satisfactory, a coverglass added.

There is hardly any limit to the size of which a section of the brain may be obtained by this method, and, if due care be taken with the planing down of the section, it is in nearly every case attended with success. In addition to exhibiting the nerve-cells, the method is useful for following the course of tracts of nerve-fibres in the nervous centres, since, owing to the depth of staining of the axis-cylinders, the nerves may be traced for considerable distances.

CHAPTER XX.

THE ORGANS OF THE SENSES—THE EYE.

THE EYELIDS, SCLEROTIC AND CORNEA.

THE study of the eye should be made as much as possible from that of the human subject, for there are slight differences in the structure of some of the parts in man and animals, and, moreover, it is on the whole easier to demonstrate the structures in the human eye. On the other hand, there is no organ which it is so absolutely essential to obtain in a perfectly-fresh condition. For this and other reasons it is scarcely possible to get material from the post-mortem room, and the only opportunities that usually present themselves occur when an eye is removed on account of some injury or disease which is confined to a particular part; the other intact portions may in such cases be available for histological purposes. In rare instances an entire healthy eye has to be removed (in operating for the removal of extensive rodent ulcers of the brow and face this may be necessary), and if the student should be able to procure such an one, the following would be perhaps the best way to deal with the excised organ with the view of making the best use of it:—As soon after removal as possible separate the eye, by an oblique cut with a very sharp knife or razor, into two halves, an anterior and a posterior; the cut to start from just behind the attachment of the iris anteriorly and superiorly, and pass downwards and backwards towards the posterior part of the organ, coming out a

little below the yellow spot and optic nerve. Then put the posterior part, after allowing the vitreous humour to fall away from the retina, into 2 per cent. solution of osmic acid, and the anterior part into Müller's fluid. The cornea is to be cut through at one place with a sharp scalpel, so that the preservative fluid may get freely into the anterior chamber.

The piece in osmic acid is left there for eight hours: it is then placed in water for two hours, and finally transferred to a mixture of equal parts of glycerine, alcohol, and water; in this it is to remain for a week or more, until wanted.

The other piece is to lie in Müller's fluid a fortnight, changing the fluid once or twice; it is then placed in water for two or three hours, then in weak spirit for a day or two, and finally preserved in strong spirit. Of the lower animals, the eyes of the pig serve best for exhibiting the minute structure, especially of the retina. In this animal the eye corresponds more closely in point of size, and approaches more nearly in structure to the human eye than that of the ox or sheep, the other animals, the eyes of which are usually readily procurable.

Preparation 1. The eyelids.—These are studied by making sections of the hardened lid across its long axis and vertically to its surfaces. The lid may be obtained from a still-born child, preferably one the blood-vessels of which have been injected. It is to be hardened in spirit and embedded, and the sections—which present no unusual difficulty—stained with logwood and mounted in dammar. In this way almost all the parts are well displayed:—the skin with its epidermis on the outer side, and with a few small hairs and sweat glands seen here and there; the mucous membrane (conjunctiva) on the inner side; the Meibomian glands cut along the length of their wide, straight duct, with their round saccules lined with epithelium cells (of a whitish glistening appearance, due to the fatty secretion they contain, and which also fills the duct); the eyelashes with their large hair-follicles and sebaceous glands; the cut ends of the very small muscular fibres of

the orbicularis arranged in groups, separated by connective tissue; and the general connective tissue which serves to unite all the parts together, and, becoming denser towards the inner surface, forms the so-called 'tarsal cartilage,' long described as composed of fibro-cartilage, in reality containing no cartilage-cells.

Preparation 2. The lacrymal gland is prepared in the same way as the salivary glands.

Preparation 3. The substance of the sclerotic.—The sclerotic is studied by means of sections made from an eye that has been hardened either in spirit or in chromic acid and spirit, the sections being stained with log-wood. There is nothing special about these preparations, and they present no particular interest. But covering the outer surface of the globe is a loose connective tissue membrane, the capsule of Tenon, composed of two apposed layers, lined by epithelioid cells which bound a lymph space, and covering the inner surface of the sclerotic is another delicate lamella of loose connective tissue, closely adherent to the fibrous substance of the coat, and of a brown appearance, due to the presence of pigment. This layer (the lamina fusca) is also bounded internally by a layer of epithelioid cells, and is separated from a similar lamella (the lamina suprachoroidea) on the outer surface of the choroid coat by another lymph space, traversed here and there by the vessels and nerves as they pass obliquely across it from the sclerotic to the choroid.

Preparation 4. Capsule of Tenon.—To exhibit the epithelioid cells of the capsule of Tenon a fresh eye is taken, and the adhering orbital fat, and everything but the insertions of the eye-muscles, removed from the globe, leaving the loose connective tissue membrane. The eye thus cleared is rinsed in distilled water, and a few drops of nitrate of silver solution are poured over the posterior part. After three minutes the silver is rinsed off again by a stream of distilled water, and the eye is placed in water in the sunlight. When sufficiently stained it is removed from the window, fastened

under water to a loaded cork by a long pin passed through the cornea, and a piece of the capsule of Tenon is dissected off the globe, floated flat on to a slide, and removed from the fluid. After the excess of water has been got rid of, the piece is covered in glycerine and examined for the epithelioid markings.

Preparations 5 and 6. Lamina fusca.—The epithelioid layer lining the lamina fusca is also prepared by nitrate of silver. A square piece of the sclerotic is dissected off from a fresh eye; the convex outer surface of the piece is then pressed in and made concave, the previously concave inner surface being made the convex one, and the piece is first dipped in water, then placed for two minutes in silver solution, then, after being again rinsed in water, transferred to spirit and placed in the light, with the inner surface or lamina fusca uppermost. After half an hour, by which time even in diffused daylight the silver will probably be reduced, although owing to the natural brown colour this cannot well be seen, it is removed, and in twenty-four hours, or when hard enough for the purpose, sections are made from the brown surface, placed in water, and mounted in glycerine. The pigment cells usually obscure the silver markings to a certain extent. This inconvenience can be obviated by using the eye of an albino rabbit. Here, moreover, the sclerotic is not too thick to admit of the piece being mounted entire in glycerine; the immersion and hardening in spirit are then not necessary, for the piece may be exposed in water to the light, and mounted without further preparation, two or three radial slits being made in it if necessary with the object of causing it to lie flat on the slide.

Besides this preparation of its epithelioid layer the lamina fusca may itself be displayed in an eye, or portion of an eye, that has been prepared with Müller's fluid. To obtain it a small piece of the sclerotic is pinned to a cork or wax-cake under weak spirit (equal parts of water and spirit); and the lamina fusca is dissected off from its inner surface, and floated on to a slide, the spirit being then

allowed to evaporate so as to leave the delicate membrane moistened only with water. The preparation may now be covered, and glycerine added at the edge of the coverglass.

Preparation 7. Sections of cornea.—The several layers of which the cornea is composed, and their relative thickness, should first be studied in sections made vertically to its surfaces. For this purpose the anterior part of an eye (pig's or ox's if a human eye is not procurable) is placed in 2 per cent. solution of bichromate of potash for fourteen days. (Müller's fluid may be used instead, but, for the cornea, possesses no advantage over the simple bichromate, although, if the retina is to be examined, Müller's fluid should be employed.) It is as well to remove the lens so that the solution passes freely to the posterior surface of the cornea. After the time specified the tissue is put into weak spirit for twenty-four hours, and then transferred to strong spirit. In two or three days more it will be ready for making sections. For this purpose a piece of the cornea is cut out and embedded in wax-mass. Very thin sections vertical to the surface are to be made, stained in logwood, and mounted in dammar. In this process a source of difficulty is sometimes met with, in the curling up of the posterior part of the section when transferred from spirit to oil of cloves, after having been stained. This can sometimes be got rid of without spoiling the section, by careful manipulation with needles; but, if it be found impossible to obviate it in any other way, the plan may be adopted of placing each section, after it has as usual been stained with logwood, and rinsed in water, in absolute alcohol for a few minutes, transferring it to a slide, and immediately covering it with the thin glass. Oil of cloves is then allowed to run under and clarify the specimen, which is prevented from curling up, in consequence of the pressure of the coverglass.

Preparation 8. Epithelium of the cornea.—The stratified epithelium covering the front of the cornea is well seen in the vertical sections, but the characters of the individual cells

which compose it must be studied in a teased preparation. For this purpose a piece of the cornea is placed in a comparatively large quantity of $\frac{1}{8}$ per cent. bichromate of potash solution, and allowed to remain in this for a week, changing the fluid once or twice during that time. Then with the point of a scalpel, or spear-headed needle, a small portion, including however the whole thickness of the epithelium, is scraped off the front, placed in a drop of distilled water on a slide, and broken up with needles as finely as possible. A piece of hair is added, and lastly the coverglass, and the specimen is then ready for examination. The cells of the various layers will be recognised by the characteristic forms they present; those of the deepest layer being in shape like a rifle-bullet, those next above cupped to receive the rounded or conical ends of the deeper cells, and the superficial layers being more flattened as they are nearer the surface. The fine ridges and furrows on many of the cells can be distinctly made out with a high power, and give a jagged contour to the cell.

To preserve the preparation permanently in glycerine it should first be stained with logwood. This is readily done by applying a drop of a very weak solution to the edge, and allowing it to diffuse under the coverglass; after a short time glycerine is added at the same edge, and gradually replaces the logwood solution, the water from which evaporates meanwhile at the other borders of the coverglass.

Preparation 9. The substantia propria of the cornea.—The fibrous structure of the substantia propria of the cornea can readily be seen by teasing out either a fresh cornea, or one which has been macerated for a while in weak bichromate of potash, or in picric acid. The lamellæ which the fibrous bundles form are apparent when an attempt is made to tear the corneal tissue, and they are well seen, cut in different directions, in the vertical sections previously made.

The corneal corpuscles are visible in the sections as mere lines, each with an enlargement in the middle, stretching across

the containing cell-space, which is fusiform in section, and is seldom filled by the included corpuscle. These appearances are best observed in the human cornea, but may also be made out in that of the pig and those of other animals. But although they look like mere lines in vertical section, they are, like most other connective tissue cells, in reality flattened out conformably to the surfaces of the lamellæ, and present when viewed flat great irregularities of form, and numerous branching proesses with which they come in many cases into connection with one another. They are best brought to view by the gold method, and, since this also serves to show the nerves, the two structures may be studied in the same preparation.

Preparation 10. Corpuscles and nerves of the frog's cornea.—The brain and spinal cord of a frog having been destroyed, the animal is laid on the table or held by an assistant, and the membrana nictitans of the eye is seized with forceps, and entirely removed by two or three snips with fine, sharp-pointed scissors. The animal is then taken up and held in the operator's left hand, the thumb pressing upwards under the lower jaw, so that the eyes are made as prominent as possible, and the point of one of the scissor blades is inserted into the globe of the eye, just behind the insertion of the glistening, yellowish iris. By a series of snips made round the eyeball at this plane, the anterior part, with the cornea, iris and lens, is severed from the posterior, and removed to a watch-glass containing salt solution. Then whilst the edge of the cut sclerotic is held by the one pair of forceps, with another pair the iris is seized close to the same spot, and is easily torn away from the sclerotic, bringing the lens with it. So that only the cornea, together with a narrow rim of sclerotic, is now left, and since it is floating in fluid it retains its convexo-concave form, and all crumpling of the tissue is avoided. The salt solution is now poured off, leaving, however, just enough to float the cornea in, and the watch-glass is filled up with half per cent. chloride of gold solution. The cornea is allowed to remain in this a full hour;

it is then removed to a beaker of water acidulated with acetic acid, and is placed in a warm place in the sunlight (see p. 79). After two days the fluid in the beaker is renewed, a teaspoonful of methylated spirit being added to prevent the growth of fungi, and in two days more the cornea may be taken out and prepared for the microscope. It is first placed in a flat dish of distilled water, and the epithelium, which is very dark and opaque, is gently scraped off the anterior surface. This done, the sclerotic rim is cut off with scissors. It is as well to change the water at this stage, so as to get rid of the *débris* of epithelium. The next process consists in the separation of the corneal substance into two, three, or more thin lamellæ. With a little practice it is not at all difficult, thin as the object already seems, by holding it down at one edge with a pair of forceps and working from the same edge with another pair, to separate a very thin lamina from the concave posterior surface, consisting of the membrane of Descemet and a delicate layer of the proper substance of the cornea with its corpuscles. This posterior lamella is not only the easiest to obtain, but is also, in the frog's cornea, the most important, for it contains the closest and finest plexus of nerves. To mount it, all that is necessary is to float it on to a glass slide, to cover the preparation, and add glycerine at the edge of the coverglass. But, since the membranous layer thus obtained has naturally the convex shape of the cornea, and it is of course desirable that it should lie flat upon the slide without creases, it is well before mounting to make three or four radial snips in it in the way shown in the adjoining cut; these will enable it to flatten out without folding, when placed on the slide and covered. Moreover it is important to examine the object with a low power previously to covering it, so that any folds of the membrane, or any foreign matter or remains of epithelium adhering to it, may be detected and removed.

The remaining anterior part of the cornea may be further separated in the same way into lamellæ, which are to be

mounted with the same precautions as the posterior lamella. It is not always easy to get them in quite so complete a layer, but for most purposes a small shred will, if equally thin, show nearly as much as an entire lamella.

In all these specimens the corneal corpuscles are stained of a violet colour, varying in tint according to the success of the preparation, their nuclei being left nearly unstained. The nerves are coloured almost black, the fibrils looking like fine wires running singly and in bundles, and provided along their course with numerous minute varicosities.

Preparation 11. The corpuscles and nerves of the rabbit's cornea.—The cornea of the rabbit, or of any other mammal recently killed, is prepared with chloride of gold in the same way as that of the frog. The eyelids are first removed, care being taken in doing this not to get the hair on to the surface of the cornea. The eye is then to be made prominent; either by an assistant who holds it firmly with forceps thrust back in the orbit, so as to seize one or other of the eye muscles near their attachment; or without an assistant by clamp-forceps, which are inserted in like manner, and by their weight force the eyeball forward without unduly compressing it. Then the cornea, iris, and lens are removed together, after cutting round the sclerotic with scissors, and are placed in salt solution, and finally the iris and lens are removed in the same way as in the preceding preparation, and the cornea is immersed in gold solution. Since it is much thicker than the frog's cornea, it should remain in the chloride of gold, which does not penetrate very rapidly—a full hour and a half—after which it is placed in acidulated water in the light, and otherwise treated in the same way as the frog cornea. But as it is not so easy to separate the mammalian cornea into lamellæ, it is better after four days to place the stained cornea in spirit for twenty-four hours, when thin sections parallel to the surface may be cut, and mounted in glycerine. Embedding in wax-mass is not necessary if the cornea is held in the left

hand, and allowed to rest over the end of the forefinger, the razor being directed *from* the operator.

Preparation 12. Isolation of corneal corpuscles.—After the corneal corpuscles and nerves have been stained with gold in this way, they can be isolated by dissolving away the intermediate substance by caustic alkali, the action being arrested before the corpuscles and nerves, which are more resisting than the connective tissue bundles, are also destroyed. With this object a part or the whole of a gold-stained cornea—whether of frog or mammal—is placed, divested of epithelium, in a watch-glass containing a strong (20 per cent.) solution of caustic potash or soda, and this is then put into a warm chamber at 40° C. At the expiration of three-quarters of an hour the tissue, which is now quite soft and pulpy, is removed with a section lifter, and placed in a vessel containing a large quantity of water faintly acidulated with acetic acid. Small portions may then be taken up and mounted, with or without further breaking up, in glycerine. The corpuscles are beautifully displayed, forming a continuous network by the junction of their branches; and nervous fibrils may be seen intercalated amongst the corpuscles, but never actually joined to, or continuous with, the cells.

Preparation 13. Nerves of the rabbit's cornea.—For exhibiting the nerves of the cornea without at the same time staining the corpuscles and the epithelium, the following modification of the gold method may be used (Klein). The cornea of a rabbit or guinea-pig is put into half per cent. solution of chloride of gold for an hour and a half. It is removed from the gold into distilled water and placed in the light (without warmth) for from twenty-four to thirty hours, or until the larger nerve trunks begin to be visible near the circumference, converging towards the centre as irregular, branching lines. When the staining has arrived at this stage, and before the corneal substance generally begins to acquire a violet appearance, the cornea is removed from the water and placed in a mixture of glycerine

and water (one part glycerine to two parts distilled water). After it has been in this for twenty-four hours, or longer, the corneal substance should be very little darker than before, but the nerves much more distinct, and on holding the cornea between the finger and thumb, and making sections from the anterior surface, including the epithelium, and a little of the substantia propria, these, when covered in the glycerine mixture, will show not only the fine and close plexus of nerves which lies immediately underneath the epithelium, but also the far more minute network of varicose ultimate fibrils which extends between the epithelium cells almost to the anterior surface of the epithelium. If not at first sufficiently evident, these intra-epithelial nerves may generally be brought more clearly into view by placing a section for a few minutes in the strong caustic potash solution. From this it is transferred by a section lifter to water, floated on to a slide, removed from the water, and covered, glycerine being afterwards added at the edge of the coverglass. To prevent the latter from pressing on the softened tissue, two narrow slips of thin glass, which may be cut with a writing diamond, are to be placed one on either side, before placing the coverglass over.

Preparations 14 and 15. The cell-spaces of the cornea.— These are shown in two ways; by the silver method, and by the method of puncture injection.

The demonstration of the cell-spaces by the silver method may be attempted in the cornea of the frog. The animal is decapitated and the brain destroyed. The eyelid is then removed, and the eye having been made prominent by the pressure of the thumb in the manner previously recommended, the epithelium is scraped off the front of the cornea with a sharp scalpel. The cornea is then rubbed with a stick of fused nitrate of silver (lunar caustic). After five minutes the surface is thoroughly washed with a stream of distilled water from a wash-bottle. The head is now placed in spirit in the light; in a short time (from a few minutes

to an hour), when the cornea is browned, the vessel containing it is removed from the window and left in a dark place for twenty-four hours. The cornea is then sliced off, placed in water, where any remaining patches of epithelium are now removed, slit in a triradiate manner, so that it may lie flat on a slide, and finally mounted in glycerine.

The cornea of mammals may be prepared with silver in a similar manner, but, being generally thicker than in the frog, it is necessary to allow the caustic a longer time to penetrate, and, in the final preparation, to prepare sections parallel to the surface, instead of mounting the cornea whole.

To inject the cell-spaces of the cornea by the puncture method, the eye of the pig or guinea-pig may be used, if a human eye is not procurable for the purpose. A solution of alkanet in turpentine is the fluid which should be chosen, and the mercurial pressure apparatus (fig. 30) is used. The tube and fine steel cannula having been filled with the alkanet solution to the exclusion of air-bubbles, the cannula is inserted obliquely into the substance of the cornea, without allowing the point to pass through into the anterior chamber. The pressure is then gradually raised to about two inches of mercury, when the red fluid should begin gradually to fill the cell-spaces, and to spread through them over a considerable part of the cornea. Indeed, if the injection is long enough continued, the fluid may extend itself even beyond the corneal margin, and may penetrate into the cell-spaces in the anterior part of the sclerotic coat. The operation may with care be successfully performed without the mercurial apparatus, using merely a Pravaz syringe. But it is difficult to avoid the production of extravasation near the point of the cannula. This does not, however, always militate against the success of the experiment, for beyond the limit of the extravasation the fluid may slowly penetrate into the cell-spaces of the tissue, and this may go on even after the syringe has been removed, especially if the cornea is cut out, laid flat on a slide, and

allowed to dry. As watery fluid becomes withdrawn from the cell-spaces in the process of drying, the alkanet solution tends to pass in to occupy its place.

Another very successful plan of inducing the injecting fluid to pass from such an extravasation into the neighbouring cell-spaces consists in gently stroking from the extravasation towards the margin of the cornea with a smooth instrument, such as a glass rod or the ivory handle of a scalpel. But if too great pressure is exerted upon the fluid it will be found that, in place of taking the closely reticulating course which it would pursue if it merely occupied the cell-spaces, the injection tends to shoot through the tissue in straight lines, which in successive planes of the corneal tissue run at right angles to one another. These lines represent the 'corneal tubes' of Bowman. Their appearance is due to the fact that the pressure exerted has been sufficient to force the injecting fluid into the interstices between the connective tissue bundles, pushing these asunder, and burrowing its way through the soft ground substance which unites the bundles and lamellæ. And, since the cell-spaces occur in this ground substance, the existence of a slight enlargement or fusiform swelling here and there on the tubes is accounted for.

These corneal tubes then are to be looked upon as purely artificial products, not corresponding with any pre-existing channels in the tissue (except perhaps when the fluid passes along the sheath of a nerve). They are always obtained when any fluid which is not able to penetrate into the cell-spaces is forced into the substance of the cornea. They are seen when mercury is injected by the puncture method, and this is how they were first obtained by Bowman; and if air be forced with a syringe into the tissue a similar effect is produced. In all cases the tubes cease abruptly at the corneo-sclerotic junction, where the connective tissue becomes denser and has a less regular arrangement.

Preparation 16. Parts at the junction of the cornea with the sclerotic.—The corneo-sclerotic junction, the ciliary

muscle, and the iris are all well seen in their relations to one another in a meridional section of the part of the eye where they are situated. The section may be made from the anterior segment which we have assumed to have been hardened in Müller's fluid and in spirit (p. 222). It is not necessary to embed this entire, but sufficient to cut out with sharp scissors under spirit a piece which includes all the parts above enumerated. The piece is first placed in oil of cloves for a few minutes; it is then transferred to cacao-butter, which is kept just melted over a sand-bath, and is allowed to remain in this for fully two hours, so that the butter may soak into every part of the tissue. Meanwhile a paper mould of the usual form is filled with the melted cacao-butter, which is allowed to become solid, and in the cake thus produced an excavation is made near one end large enough to receive the piece to be embedded, which is placed in the hollow in such a position that radial sections vertical to the surface may be made, and the excavation is then filled up with melted cacao-butter. This in hardening sets into one piece with the rest of the cake, and in cutting the sections the advantage is gained that all the parts have exactly the same density and offer a uniform resistance to the razor. Much thinner sections may therefore be made by this method than by any other. The sections, as they are obtained, are placed in oil of cloves, which slowly dissolves the cacao-butter out from the tissue: the process can be accelerated by slight warmth. They are next placed in alcohol to remove the oil of cloves; then for a few minutes in Kleinenberg's logwood; and are finally passed again through alcohol and oil of cloves, to be mounted in dammar varnish in the usual way. Few specimens better repay the trouble of preparation than these. The cornea, sclerotic, iris, choroid, ligamentum pectinatum, canal of Schlemm, ciliary muscle, both radial and circular, and even the ora serrata and pars ciliaris of the retina, are all exhibited with the greatest clearness in a successful section, and their structure and relations may be advantageously studied.

THE CHOROID AND IRIS.

The choroid coat is to be prepared from an eye hardened in Müller's fluid. Besides the main substance of the coat containing the larger bloodvessels, the lamina suprachoroidea, the chorio-capillaris, and the membrane of Bruch, should all be separately displayed. The posterior attachment of the fibres of the ciliary muscle, and the gangliated plexus of nerves which is found in the neighbourhood of its posterior attachment, will be exhibited with the lamina suprachoroidea.

Preparation 17. Ciliary muscle and lamina suprachoroidea.—To prepare these, the anterior part of the eye is to be pinned down under spirit, and the cornea and sclerotic cut away at one part, when the radiating fibres of the ciliary muscle will be seen passing meridionally from their origin opposite the attachment of the iris, and forming a layer which becomes gradually thinner as it extends backwards and finally ceases in the superficial part of the choroid. A small piece of the muscle is seized with sharp forceps near its origin, and, by carrying the instrument slowly backwards, is gradually torn away from the rest. It will be found that the shred which comes away generally spreads out posteriorly into a very thin membranous lamina, this being in fact a piece of the lamina suprachoroidea into which the superficial fibres of the ciliary muscle are inserted. A considerable length may be torn off in this way, and the piece so obtained is to be floated directly on to a slide, which is dipped for the purpose into the spirit. It must be moved with great care, so as to avoid folds or creases. The slide is quickly wiped free of spirit, with the exception of that which immediately moistens the specimen, and a drop of freshly-filtered logwood is placed upon the tissue, and allowed to remain on it for ten minutes. The staining solution is then poured off, and the remains of it are removed by allowing a

drop or two of water to flow gently over, without disturbing the position of the membrane. Finally a coverglass is laid on, and a drop of glycerine allowed to run in at the edge of the coverglass.

The preparation so obtained is, if successful, a very striking one. Besides the branched pigment cells of the choroidal tissue, and a certain number of cells, similar to white blood-corpuscles, on the surface of the membrane, a number of large, round or oval nuclei are seen in the lamina, which are apparently devoid of cell-body. These are in reality the nuclei of epithelioid cells which bound the *lamina supra-choroidea* externally, and serve as part of the lining of the lymphatic space which lies between this and the lamina fusca of the sclerotic. Their outlines cannot be brought to view without the aid of nitrate of silver, and the cell-bodies are too delicate and transparent to be shown by the present mode of preparation. A large number of elastic fibres are also seen on the membrane, especially at the terminations of the fibres of the ciliary muscle, where they appear to come into relation with the ends of these, an elastic fibre passing for a certain distance along each side of the muscular fibre, and seeming to serve in this way for its attachment. The involuntary fibres are particularly well shown, their nuclei being conspicuously stained by the logwood; many of the bundles terminate in peculiar tufts, from which the fibres radiate in all directions. It may happen that the preparation includes one of the long ciliary nerves; this, as it coursed forward to enter the ciliary muscle, having been torn away with the shred of membrane. If so, it may be followed with the microscope and its branches traced amongst the bundles of muscular fibres, forming a plexus with those of the other nerves. In tracing the branches characteristic ganglion-cell enlargements will here and there be found interpolated in the course of a nerve fibre.

Preparation 18. Vascular layers of choroid and membrane of Bruch.—As seen in the preparation just described, it is

easy to detach the lamina suprachoroidea from the rest of the choroid. The other three parts are more difficult to separate, and their complete isolation may require considerable time and patience. But for demonstrating their structure it is not absolutely necessary for them to be completely separated as distinct membranes; it is sufficient if, in a piece which includes all, one or other is left projecting at the edge, so as in this way to be seen distinct from the other layers. But before commencing the attempt at separation, the hexagonal pigment cells, which belong to the retina but frequently adhere to the inner surface of the choroid, must be entirely removed by gently brushing that surface with a hair pencil. The separation and brushing are performed under fluid (spirit), and will be much facilitated by the use of a dissecting lens.

Preparation 19. The musculature of the iris.—The circular and radiating plain muscular fibres of the iris may be demonstrated in the albino rabbit. The eye is cut in half and the anterior part placed in spirit for a day or more. Then the lens is removed, and a segment of the iris—including its whole width, from the pupillary aperture to the ciliary processes of the choroid—is cut out and placed in dilute logwood. When sufficiently but not too deeply stained, it is put into water for a minute or two to remove the excess of staining fluid, then passed through spirit and oil of cloves, and finally mounted in dammar, with the posterior surface uppermost. The thick ring of the sphincter is easily seen in these preparations, and also the interlacing bundles of plain muscular fibres of which the dilatator is composed; they may be observed to bend round near the pupil, and take the direction of and blend with those of the sphincter. At the circumference of the iris also, a similar bending round of the radiating fibres is observed.

Preparation 20. Human iris.—Although, in consequence of the presence of the uveal pigment, more troublesome to prepare, it is nevertheless desirable to make a similar preparation of the human iris, for the musculature is somewhat

different, the dilatator forming a uniform thin expansion, which covers the posterior surface immediately under the pigmented epithelium, and not distinct radially arranged interlacing bundles with intervening meshes as in the rabbit. The specimen may be made from an eye that has been in Müller's fluid or bichromate of potash (2 per cent.), and subsequently in spirit. A piece is cut out as before, and is treated in a similar way, except that before being stained the pigment is brushed completely off the posterior surface with a stiff camel-hair brush. This must be done under spirit, and of course very carefully so as to avoid tearing the tissue; during the operation the iris is examined now and then with a low power, to determine when all the pigment is removed. It is difficult to prevent some of the pigment granules from still sticking to the surface, but, as they tend for the most part to adhere along the lines of junction of the fibre cells, their presence does not spoil the object of the preparation, for the dilatator fibres are if anything better displayed.

THE RETINA.

Preparations 21 and 22. Sections of the Retina.—It will be well to study the general arrangement of the several layers of the retina by means of sections, before its constituent elements are observed isolated. Two methods of hardening may be recommended, viz. by Müller's fluid and by osmic acid, the process being in both instances completed by spirit. If possible to obtain it perfectly fresh and healthy, the human retina should always be used; if not so obtainable, that of the pig is preferable to the retina of most other animals, and in the following preparations it will be assumed that the eyes of that animal are employed.

To harden the retina in Müller's fluid it is better to keep the bulb whole, so that the membrane remains supported by the vitreous humour. But to let the fluid in readily two or three cuts must be made in the sclerotic with a razor,

or a piece even be cut out at one or two places. The eye is then dropped into a relatively-large quantity of Müller's fluid, and allowed to remain in it for a week, the fluid being stirred at intervals; the globe is then cut across, and left for another week in the liquid, after which time it is transferred first for twenty-four hours to weak, and then to strong spirit. In another day or two it is ready for the preparation of sections. Small pieces from different regions may be taken, but as they are all treated in the same way, they may be described as one :—

The staining of the tissue is first effected by placing the piece for twenty-four hours in alcoholic logwood (Kleinenberg's). From this it is transferred to spirit, then embedded in wax-mass. It is better so to place it in the embedding tray that the sections shall be both vertical and meridional; since, made in this way, they will take the general course of the fibres of the optic nerve. The sections cannot be made too thin, but they should be complete, that is to say, they should include all the layers of the membrane. One or two of the thinnest are to be mounted in glycerine ; the others may be transferred from the spirit to oil of cloves, and mounted in dammar. In these preparations the nerve fibres, the Müllerian fibres, the inner and outer granules, and in fact the layers generally, are all well displayed, but the rods and cones are much altered, and the natural form and appearance of the other elements is by no means well preserved.

In this respect the osmic method yields far better results. The bulb in this case is cut open, and either the whole or a piece only of the retina is taken and placed in 2 per cent. solution of osmic acid. Here it is left for several hours, and then, after being an hour or two in water to get rid of the excess of osmic acid, it is transferred to alcoholic logwood for twenty-four hours. From this it is placed in strong spirit, where it may be left until wanted. It is well to embed the piece by the cacao-butter process, since by

hardly any other method can sufficiently thin and complete sections of a retina that has been hardened in osmic acid be obtained. For this purpose it is as usual first placed in oil of cloves, and then allowed to soak for half an hour or more in cacao-butter, which is kept just melted by a gentle heat. When completely permeated the piece of membrane is placed in the proper position in a cavity scooped out in a cake of cacao-butter, and after it has firmly set, sections may without difficulty be made of exquisite fineness. They are placed in oil of cloves—in winter it must be very slightly warmed—which speedily dissolves out the infiltrated cacao-butter, and they may then be mounted in dammar. But it is better to transfer them from the oil of cloves to spirit again, and afterwards to mount them in glycerine, since in this the details can be made out with greater readiness than in the more highly refracting dammar. For the same reason a saturated solution of acetate of potash is sometimes recommended as superior to glycerine for mounting osmic preparations, especially of the retina.

Preparations 23, 24, and 25. Isolation of the retinal elements.—Various processes are employed for macerating portions of the retina in order to obtain its elements, either in a completely isolated condition or still partially connected with one another. It will be best, in the first place, to attempt the separation with a piece of retina which has been in 2 per cent. osmic acid for six or eight hours. It must of course be put in perfectly fresh, and after the time mentioned it is placed in water for twenty-four hours. It is then allowed to macerate for a few days in a mixture of glycerine, alcohol, and water (glycerine one part, alcohol one part, water two parts), after which a minute portion is to be carefully broken up with fine needles in a drop of weak glycerine, and, a piece of hair having been added, the coverglass is superposed, and the preparation examined with a dry objective and, after fixing the coverglass, with an immersion. The effect of the osmic acid is to preserve the more readily alterable

elements, such as the rods and cones and their nuclei, in a condition as nearly as possible approaching that which they possess during life.

Other portions of fresh retina are to be placed, one in $\frac{1}{8}$ per cent. bichromate of potash solution (for a week), and the other in 10 per cent. chloral hydrate solution (for two or three days). The portions so macerated are to be teased out in a drop of their respective solutions, the usual expedient being adopted of obviating the pressure of the coverglass by a hair. In the one treated with bichromate the Müllerian fibres, and in fortunate preparations the ganglion cells with their processes, may be well seen and isolated. The granules of the inner nuclear layer are also frequently obtained with one or both processes extending from their opposite poles, but the rods and cones are for the most part much altered and granular in appearance, so as hardly to be recognisable.

In the chloral hydrate preparation, the last-named elements are much better preserved. The external segments of the rods, which even in the osmic preparation tend for the most part to become altered, may by this method be frequently seen almost unchanged, except that the transverse striation, which indicates their discoid formation, is often well marked. Most of the other structures are also well preserved, and the Müllerian fibres and ganglion cells are sometimes better seen than in the bichromate preparation. Unfortunately neither the bichromate nor the chloral specimens can be well preserved without deterioration.

Preparations 26 and 27.—The study of the retina cannot be considered complete until the elements have been examined in the fresh, unaltered condition. A small piece, taken from an eye still warm from the animal, should accordingly be broken up as rapidly and finely as possible in a drop of serum, or, failing this, in a little vitreous humour. A modification of this very simple method consists in allowing a small piece to macerate for two or three days in weakly

iodised serum (see p. 45) before attempting the dissociation, which can then be more readily effected.

Preparation 28.—The hexagonal pigment of the retina is seen in most of the teased preparations above described. In eyes that have been hardened in Müller's fluid the layer often separates in flakes of varying size, and nothing is simpler than to remove such a piece with a section lifter, and mount it in glycerine, so as to exhibit the pavemented appearance which the cells present.

Preparation 29.—On the opposite surface of the retina also a mosaic-like appearance can be demonstrated by the aid of nitrate of silver, but it is much more irregular, and does not depend upon the presence of epithelium cells, but upon the flattened-out ends of the Müllerian fibres. To show this appearance, a fresh eye is cut in half transversely, and the vitreous is shelled out from the posterior half; this is then rinsed in distilled water and transferred to nitrate of silver solution ($\frac{1}{2}$ per cent.). After a minute in this it is again rinsed in distilled water, and exposed in water to the light. When the surface is browned it is removed, a piece of the retina is cut out under water, floated on to a slide with the inner, brown surface uppermost, and the water is drained off and replaced by glycerine, when the preparation can be covered and examined, care being taken not to let the cover-glass press upon the specimen.

Preparation 30. The Retina in the lower Vertebrata.—The structure of the outer segments of the rods, which it is difficult to make out in mammals, can be seen easily, even with an ordinary high power, in the retina of amphibia. With this object the eye is removed from a recently killed frog, cut across, and a small portion of the retina is quickly broken up in vitreous humour. A piece of hair having been added, the preparation is covered and examined. Almost everywhere the field of view is strewed with large, clear, rod-shaped structures, some straight, but many of them bent and curved in different directions, and exhibiting a distinct transverse striation, or even a tendency to split up into a number of superimposed discs, this tendency increasing as the preparation is longer made. Some of them

have what looks like a small appendage jointed on at one end, but this is really the comparatively small inner segment of the rod. The cones are also very small in comparison, and on that account may at first be missed; they are distinguished by the possession at the apex of the inner segment of a small, bright, fatty globule, often of a yellow colour. Most likely a portion of the hexagonal pigmented epithelium will have come away with the rest of the retina, and in consequence of the rupture of some of the cells the preparation will be strewed with pigment granules which, like all minute granules suspended in fluid, exhibit very strikingly the Brownian molecular movement. Some of the pigment cells may be observed intact, either isolated or in patches. If seen in profile, it may be noticed that near one surface (the outer) the cell is almost entirely free from black pigment, while from the other fine streamers of the cell-protoplasm, dotted with pigment granules, extend. In their natural position these pass between and amongst the outer segments of the rods.

Preparations 31, 32, and 33.—The retina of a bird, of a reptile (tortoise), and of a fish are to be teased out fresh in vitreous, in the same way as that of the frog. The chief points of interest in these preparations are the ellipsoid or lenticular bodies in the inner segments of the rods (bird and amphibian) and cones (bird, reptile, and amphibian), the bright, fatty globules of different colours in the inner segments of the cones in the tortoise and bird, and the twin or double cones, especially large in the fish's retina. The various other points in which the retina in these animals differs from that of mammals, may be studied by employing the same methods of preparation as for the mammalian retina.

THE LENS AND VITREOUS HUMOUR.

Preparation 34. The Lens Fibres.—The following will be found the best mode of isolating the fibres of the lens, as well as for showing their arrangement. Take the fresh eye of any animal—that of the ox or sheep for example—and cut it across into an anterior and a posterior half. Place the anterior part, having removed what remains of the vitreous humour, in $\frac{1}{8}$ per cent. solution of bichromate of potash. Then scratch through the posterior capsule, which is readily ruptured

and curls away from the lens proper. This can easily be shelled out, and is left in the fluid, the remainder of the eye being rejected. The lens is allowed to remain in the bichromate for two or three days, being merely turned over once or twice. It will be found that its substance tends both to separate along the radiating lines which mark the planes of junction of the ends of the fibres, and also to peel into concentric lamellæ like the coats of an onion; and, if a piece of one of these lamellæ is taken up with the forceps, it will tear in the direction of the fibres from one of the planes of junction of the anterior surface to the corresponding plane of the posterior. The fibres can be readily separated with needles in a drop of the bichromate solution. For this purpose portions should be taken both from the superficial and from the more central parts of the lens. In many of the superficial fibres a round or elongated nucleus may be detected at one part, and since the nuclei of adjacent fibres are met with in about the same region, when a number of fibres are seen together the nuclei lie in an irregular row. The riband-like shape of the fibres may be made out at parts where they are turned over so as to be seen edgeways.

Preparation 35. Sections of the Lens.—For cutting sections of the lens it is best to harden it in Müller's fluid. The whole anterior half of an eye should be put in this, the cornea having been partly removed so as to enable the fluid to get freely to the front as well as to the back of the lens, but the capsule is not to be ruptured. After two or three weeks, vertical sections may be made, one of which should pass through the centre from before back, and should be as complete as possible. They are to be mounted in glycerine. The lens must not be put in spirit, or at all events not in strong spirit, to complete the hardening, for this renders the tissue, especially the central parts, quite hard and horny, and the outlines of the fibres become obliterated.

Preparation 36. The Epithelium of the lens-capsule.—This may have been seen in the antero-posterior section as a

single row of nucleated cells, lying immediately behind the anterior part of the capsule. To show it on the flat it is to be stained with nitrate of silver. With this object a lens still enclosed in its capsule is removed from a fresh eye, and, after having been rinsed in distilled water, transferred for five minutes to $\frac{1}{2}$ per cent. nitrate of silver solution. It is then again washed with distilled water, and placed in the light in weak spirit (equal parts spirit and water). When brown it is removed from the light, and after twenty-four hours the substance is hard enough to allow tangential sections to be made from the anterior surface, which shall include the capsule, the epithelium, and the parts of the lens substance immediately subjacent to this. The sections are mounted in glycerine with the brown surface uppermost: and through the elastic capsule, which is not distinctively stained, the outlines of the epithelium-cells are clearly seen. At places the silver solution may have penetrated to the superficial lens fibres, and will be found to have stained the cementing substance between them.

Preparation 37. The zonule of Zinn and the hyaloid membrane of the vitreous humour.—Take the anterior half of the eye (preserved in spirit) of an albino rabbit, and having pinned the cornea downwards on a loaded cork under spirit, and removed the remains of the vitreous humour, gently seize the lens with fine forceps, and draw it away from the iris. In doing this it will drag with it the suspensory ligament, the zonule of Zinn, and the part of the hyaloid membrane continuous with this, so that the separated lens appears girdled by a delicate, somewhat crumpled-looking, membranous zone, closely adherent at its inner border to the equator of the lens, and bounded outwardly by a ragged margin,—the torn edge of the hyaloid. Cut out with fine scissors a segment of this zone, including its whole breadth, and with a section-lifter transfer the piece so removed to logwood solution (Kleinenberg's). When sufficiently stained—and it stains very readily—transfer it to a dish of water, and from this float

it on to a slide, avoiding all creases except of course the natural ones of the zonule. It may then be covered, and the water in which it is mounted replaced by glycerine. Or, instead of placing it in the water, it may be transferred from the logwood to spirit, and then passed through oil of cloves and mounted in dammar. These preparations exhibit well the folds and striations of the zonule, and the rounded corpuscles, like white blood-corpuscles, which are dotted here and there over the surface of the hyaloid.

THE BLOODVESSELS OF THE EYE.

For the demonstration of the bloodvessels the head of an albino rabbit should be injected, a cannula being placed in each carotid, and the two cannulas connected to the arms of a Y-shaped tube, the stem of which is brought into communication by an india-rubber tube with the injection bottle. After the blood has been driven out of the vessels before the flow of injection fluid, the neck of the animal, just below the place where the cannulas are inserted, is surrounded by a loop of wire, which is drawn as tightly as possible to prevent the escape of the injection; and the pressure is then raised to about four inches of mercury and kept there for some minutes, so as to make certain that all the bloodvessels shall be completely filled. The whole is then allowed to stand and become cold, that the gelatine may set, after which the eyes are to be carefully excised, and placed in weak spirit. In twenty-four hours this may be increased in strength, and in another twenty-four or forty-eight hours they are to be placed in strong spirit. When they have been in this a day or two the following parts may be prepared:—

Preparation 38. The conjunctival vessels, and the subconjunctival vessels of the sclerotic.—By making a tangential section from the region of the corneo-sclerotic junction, and after passing the piece so obtained through oil of cloves,

mounting it in dammar with the outer surface uppermost, the distribution of the vessels, both in the conjunctiva and, by focussing more deeply, those in the sclerotic at and near the margin of the cornea, is exhibited. Another plan consists in cutting away a small piece, including the whole thickness of both cornea and sclerotic, and mounting in a similar way. The thickness and irregularity of the piece so obtained is a disadvantage, but, on the other hand, the canal of Schlemm and the other venous sinuses may be observed, if the injection has been a successful one, by focussing still lower than for the looped vessels of the sclerotic.

Preparation 39. Vessels of the choroid and iris.—One of the two injected eyes is to be divided by an antero-posterior cut with the razor into a right and a left half. One of the two halves, the one which does not include the attachment of the optic nerve, is first taken, and the vitreous, retina, and lens removed, so as to clear the inner surface of the choroid and iris. The last-named are next to be separated as one piece of membrane from the sclerotic. The piece so obtained is then to be again divided into two, by another antero-posterior cut with the scissors, and the resulting halves are to be mounted, after passing as usual through oil of cloves, in dammar, the one with the inner and the other with the outer surface uppermost. Each includes, of course, the fourth part of the choroid coat with some of the ciliary processes, and a piece of the iris; and with a low power the course and disposition of the bloodvessels in these parts can be readily followed. Besides these comprehensive preparations, separate ones may be made from the other half of the eye of a portion of the iris (this is rendered more instructive by lightly staining it with logwood), and one or two of the ciliary processes snipped off with sharp scissors, and mounted so as to be seen in profile.

Preparation 40. The vessels of the retina.—If the other injected eye be cut into an anterior and a posterior half, and the posterior part is examined after removal of the vitreous

humour, the bloodvessels will be seen spreading out from the centre of the colliculus of the optic nerve. To exhibit their finer distribution in the retina, a piece is mounted flat in dammar without previous staining, while, to show the extent of their distribution in the retinal layers, vertical sections, which need not be very thin, may be made from a piece embedded in wax-mass in the ordinary way, and similarly mounted, without staining, in dammar.

CHAPTER XXI.

THE EAR.

The only parts of the ear which require special directions for their preparation are the semicircular canals and the cochlea.

Preparations 1, 2, and 3. The semicircular canals.—To study the structure of the membranous semicircular canals, those of the cartilaginous fishes, e.g. the skate, are chosen. The skull, which can be readily cut with a scalpel or strong pair of scissors, is opened quite anteriorly, where it is occupied merely by a quantity of cerebro-spinal fluid, and the opening is extended backwards by removing the roof bit by bit, until the whole of the upper surface of the brain is exposed. Two thick cartilaginous masses will be seen, one on either side, near the posterior part; the large auditory nerves pass through a foramen in each into their interior. These masses enclose the membranous labyrinth, consisting in these animals of utricle, saccule, and semicircular canals, all of large size, and contained in corresponding cavities and canals, in the substance of the cartilage, but of which no trace can at present be made out. If, however, horizontal slices are made with a scalpel, one of the canals will soon be exposed, and this can then be followed in both directions, cutting the cartilage away so as to expose the included membranous canal in its whole length. It will be found to lead at either end into a large membranous bag—the utricle—with which the two other canals also communicate, and from which they can be traced in the same manner. Besides the utricle, there is another smaller mem-

branous bag—the saccule—and both contain a white, pasty, cretaceous, otolithic mass, which lies over the part to which the nerve proceeds. Near one of the attachments of each semicircular canal to the utricle is its dilated part, or ampulla, and a branch of the auditory nerve may be seen proceeding to each of these, and terminating abruptly in a forked thickening, which indents the membranous wall and lies transversely to the axis of the ampulla.

The three ampullæ, and the adjacent portions of the semicircular canals, are now to be removed from the cavities containing them, and are to be placed, one in a weak solution of chromic acid ($\frac{1}{6}$ per cent.), one in osmic acid (2 per cent.), and the third on a slide in a drop of endolymph obtained from the cavity of the utricle. The piece in chromic acid is transferred to weak spirit after three days, and in twenty-four hours more to strong spirit. After another day or two it may be placed in Kleinenberg's logwood for several hours, and then embedded either in wax-mass in the ordinary way, or by the cacao-butter process. Sections are to be made both of the semicircular canal proper, and of the ampulla, opposite to and including the entrance of the nerve; and the sections are passed through oil of cloves, and mounted in dammar. The piece in osmic acid is transferred after twenty-four hours, first to water for two or three hours, and then to spirit; after a day or two in this it may be placed in oil of cloves, and subsequently permeated with and embedded in cacao-butter. The sections, after the cacao-butter has been removed by oil of cloves, and this again by spirit, are finally mounted in glycerine. The third piece, especially the part where the nerve enters, is broken up at once in the drop of endolymph, and examined with a high power, with the view of observing the two kinds of epithelium cells—columnar and spindle-shaped—which occur here, and the stiff, hair-like projections which are attached to them. The demonstration of these structures presents, however, the greatest possible difficulty.

Preparation 4. Sections of the cochlea.—On account of the thinness of its osseous parietes, the ease with which it may be obtained separate from the surrounding bone, and its comparatively large number of spiral turns, the cochlea of the guinea-pig offers far greater facilities for study, and especially for the preparation of sections, than that of any other animal. The following is the mode of finding and procuring it:—In the recently-killed animal the aperture of the mouth is prolonged backwards on either side, by cutting through the cheeks and temporal muscles with strong scissors. The lower jaw is then seized and forcibly torn away from the rest of the head, so that the base of the skull is exposed. Here will be seen on either side, just behind the fossa for the articulation of the condyle of the jaw, a large white bony projection—the tympanic bulla. This is not yet to be opened, but the cartilaginous external auditory meatus is first cut through, and with the aid of bone-forceps or strong scissors, the bulla in question, together with the petrous bone to which it is attached, separated from the rest of the skull. In a young animal this can be very readily effected, simply by inserting a strong blunt instrument into the base of the skull just in front of the bulla, and using it as a lever, raising the bone and forcing it away from its attachments. The bones of either side being thus removed, the adhering soft parts are cleared away, and the bulla is broken open at its most prominent part. On now looking into the cavity there will be noticed, on one side the delicate tympanic membrane stretching over the end of the external meatus, with the handle of the malleus attached to it, and on the opposite wall a well-marked conical projection, which there is no difficulty in recognising as the cochlea; indeed, its bony wall is so thin that it is possible to count the number of turns (four) which it presents. By cutting the bulla round with strong scissors, the two parts—one including the tympanic membrane, and the other the cochlea—are separated from one another, and the membrane part

may at once be dropped into weak spirit and put aside to be subsequently stained and mounted. From the other part as much as possible of the substance of the petrous bone is snipped away, bit by bit, from around the base of the cochlea with scissors or bone forceps, but great care should be taken in approaching the cochlea itself, as this is very readily split. When the surrounding bone has been in this way removed, the cochleas are dropped into Müller's fluid. In this they are to be left for a week or fortnight—even a longer immersion will do them no harm—until the soft structures in the interior are somewhat hardened. The process is then completed, whilst the bone is at the same time softened by transferring the cochleæ to a saturated solution of picric acid. When the bone is completely softened, a process which is much facilitated by frequent disturbance of the fluid, the cochleas are transferred to weak spirit (half water), and in twenty-four hours more to strong spirit. After being in this for two or three days they are ready for embedding. The best mass to use for this purpose is a mixture of wax and cacao-butter, equal parts of each. One of the prepared cochleas is fixed by a pin in the embedding box in such a position that the plane in which the sections are made shall be exactly parallel with the axis of the cone which the cochlea forms, and the cacao-butter and wax, previously melted and thoroughly mixed, are poured into the mould. When the mass has become hard, sections may be made; the first ones will include only the large basal turn of the cochlea, then the higher turns will all be included in succession, until at last the modiolus is reached. All the sections which have been made up to this point may be rejected. Great care must now be taken to make thin and complete sections of this central part. They will of course be triangular in shape, with a rounded apex; the section of the modiolus occupies the axis from the base to near the apex of the triangle, and on either side are the sections of the successive spiral turns. Not more than three or four complete axial sections can be obtained from

each cochlea. The razor and the surface of the cake should be wetted with oil of cloves instead of spirit. It may be applied by means of a large camel-hair brush, and its use is that it renders the parts more coherent, and tends to prevent the organ of Corti from breaking away from the basilar membrane, which it is otherwise apt to do. The sections are examined, unstained, with a low power, and if the organ of Corti is sufficiently complete they are at once transferred to an alcoholic staining fluid. One of the best is a weak solution of acetate of rosaniline, but if this is not at hand, some other aniline dye, such, for example, as eosin (see Appendix), may be employed. When stained the sections are again placed in oil of cloves, and finally mounted in dammar.

Preparations 5 and 6. Teased preparations of the cochlea.—Successful sections will show the general position and relations of the rods and other parts, and to a certain extent the individual elements. But only a profile view can in this way be obtained, and since the minute structure of the elements composing the organs of Corti can only be properly seen when isolated, it is necessary to prepare other cochleas with this object in view. Another animal is accordingly sacrificed, and the cochleas removed as before. One is placed in a 2 per cent. solution of osmic acid: the other in a $\frac{1}{4}$ per cent. solution of bichromate of potash; but, before dropping them into their respective fluids, the bony wall must be scraped through here and there with a scalpel, so that the fluid shall at once penetrate to the interior of the turns. After two days the cochleas may be further prepared in the following way:—

The uppermost turn is broken or snipped off with scissors, placed in a drop of water on a slide, the shell of bone which forms the cupola and outer wall removed, and the piece of lamina spiralis examined with a low power (without covering the preparation) in order to learn to recognise the structures which lie on it. The glass slide is then removed to the dissecting microscope, and with very fine needles the lamina

spiralis is separated from the columella, which is then rejected. Next all the parts on the lamina, but especially the row of rods of Corti, to which the hair-cells as a rule cling, are broken up finely, but at the same time slowly and carefully, the preparation being examined now and again with the highest power which it is safe to use without a coverglass. One of the chief difficulties is apt to arise from portions of the tissue sticking to the needles: if this is the case, pieces of glass rod drawn out to a fine point may be substituted. When the more important parts have been broken up pretty completely, any thick pieces of tissue unimportant to the present observation, such as bits of bone, or periosteum, bundles of medullated nerve fibres, &c., should be picked out, and then a coverglass laid on and the preparation examined. To preserve either preparation permanently glycerine may be allowed to diffuse in at the edge of the coverglass; but the bichromate specimen should first be treated with a drop of carmine solution, so that the elements are somewhat stained, otherwise they will be rendered too transparent by the glycerine.

In this way a number of specimens may be obtained from each cochlea—proceeding from above, down, and preparing turn after turn; and careful sketches should be made of the different structures met with, and their arrangement with regard to one another. It will be found that the osmic preparations serve best for showing the lamina reticularis and the lamina basilaris, and the bichromate preparations for the hair-cells and the membrana tectoria; the other structures are almost equally well seen in both kinds of preparations. The large fat droplets in some of the epithelium cells of the uppermost turn are peculiar to the guinea-pig, as is also the arched projection—seen in the sections—at the part where these cells are found. The fat drops are stained black in the osmic preparation.

THE OLFACTORY ORGAN.

Preparations 1–3.—Small pieces of the upper turbinate bones, or from the upper (olfactory) region of the septum nasi, from the dog or rabbit, are placed, one piece in $\frac{1}{6}$ per cent. chromic acid solution, a second in $\frac{1}{4}$ per cent. bichromate of potash, and a third in 1 per cent. osmic acid. The one in chromic acid may remain a week, when it is transferred to weak spirit and in twenty-four hours more to strong spirit. After a day or two in this, vertical sections are prepared from it.

The other two pieces are examined after forty-eight hours' maceration, small pieces of the mucous membrane being teased out so as to isolate the epithelium cells (both columnar and spindle-shaped), and if possible, especially in the osmic preparations, to study the connection of their branching lower ends with subjacent structures. These preparations can be preserved with glycerine, the bichromate one being stained with logwood.

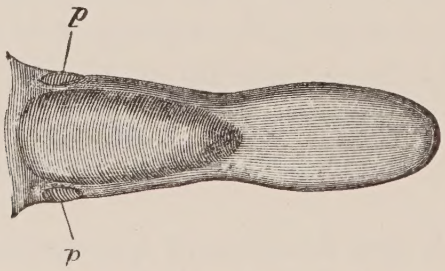
Preparations 4 and 5.—Teased preparations should also be made of the olfactory mucous membrane of the frog or newt. Having cut off the head of the animal, and slit up the nostrils with fine scissors, place it in a quantity of $\frac{1}{4}$ per cent. solution of bichromate of potash. After two days preparations of the epithelium from both the anterior and posterior part of the passage may be made. The cells are obtained with the greatest ease, by scraping the mucous surface with the point of a scalpel, and shaking out the material in a drop of water on a slide. A piece of hair is added, and the preparation covered and examined. In the portion obtained from near the anterior nares ordinary, columnar, ciliated epithelium cells will be seen. In that from the true olfactory part the cells, although many of them are of a columnar form, are destitute of cilia, and in addition to the columnar elements spindle-shaped (olfactory) cells are met with which are pro-

vided with a bunch of stiff-looking, hair-like processes, resembling the similar appendages of the auditory epithelium.

THE GUSTATORY ORGANS.

For studying the taste-buds, the foliated papillæ which are found on either side of the base of the rabbit's tongue are used. To obtain them the tongue is cut out entire from the recently-killed animal, when the little oval patches marked with transverse ridges may readily be found (fig. 32, *p*, *p*). They are snipped off with curved scissors, and one is dropped into a mixture of equal parts of spirit and $\frac{1}{2}$ per cent. chromic acid, and the other is placed in 1 per cent.

FIG. 32.



Tongue of rabbit, seen from above.

p, *p*, Papillæ foliatæ.

osmic acid. After two days the spirit and chromic mixture is to be exchanged for strong spirit, and in twenty-four hours more, the piece of tissue may be embedded so as to cut sections, which should be as thin as possible, vertical to the surface of the mucous membrane, and across the direction of the ridges. The sections are stained and mounted as usual.

The piece that was placed in osmic acid may be used at the end of forty-eight hours. In the first place two or three sections are to be obtained like those made from the other piece, without however embedding the tissue, but simply holding it in the fingers or in a piece of split cork. One

such section is to be placed in a drop of water on a slide, and an attempt made with needles, under the dissecting microscope, to separate some of the taste-buds from the surrounding epithelium. For this purpose the needles must be very fine, sharp, and clean, and the lens used as high as is consistent with convenience of manipulation. When one or more taste-buds have been thus separated, the rest of the section is removed, and the isolated buds are broken up as completely as possible into their constituent cells. The specimen may then be covered, and a drop of glycerine allowed to diffuse in under the edge of the cover; after which an examination of the preparation may be made, at first with the ordinary high power, and afterwards with an immersion objective.

APPENDIX.

Method of measuring an object under the microscope.—If while the one eye looks down the tube the other is allowed to remain open, an image of the object will appear projected on the table at the side of the microscope, and it is not difficult to mark off, upon a sheet of paper placed here, the points between which the measurements are to be taken. The preparation is then removed, and a stage micrometer is substituted for it, the parts of the microscope being left in the same condition as before. The *stage micrometer* is a glass slide on which fine equidistant parallel lines have been ruled with a diamond. The distance between the lines is marked on the slide; it is generally either the $\frac{1}{100}$th and $\frac{1}{1000}$th part of an inch, or the $\frac{1}{10}$th and $\frac{1}{100}$th part of a millimeter. The lines are observed with the microscope in the same way as the object, and their image can of course be similarly projected upon a sheet of paper and there marked down. The distances between the lines being known, it is easy, by comparison of the two markings, to find out the distance between the opposite points of the object.

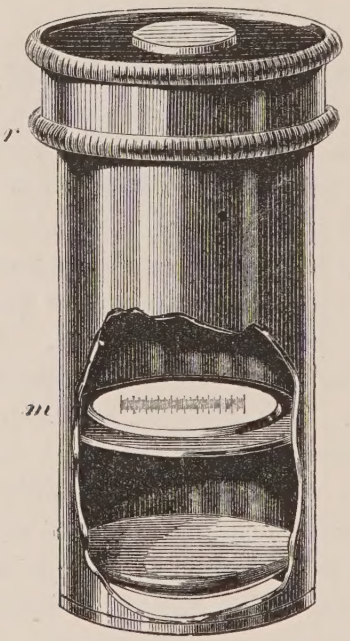
Some microscopes are provided with an *eye-piece micrometer* (fig. 33). This is an ordinary ocular with a flat piece of glass (*m*) having a scale ruled upon it by a diamond, inserted between the field-glass and eye-glass. The value of the divisions of the scale should be determined once for all for each objective by observation of a stage micrometer (see fig. 34), the tube of the microscope being fully drawn out, and should be marked on the ocular; and in subsequently using it for measurement all that is necessary is to see how many divisions of the scale the object under examination covers. Thus, supposing it had been found by examination of a stage micrometer that with the high power objective and the tube drawn out each division of the eye-piece micrometer was worth $\frac{1}{1800}$ inch, any object which when viewed by the same objective and length of tube took

up three divisions of the eye-piece micrometer would measure $\frac{3}{1800}$ths or $\frac{1}{600}$th of an inch.

The advantage of the eye-piece micrometer is that when its values are once ascertained the size of an object can be read off at once.

Determination of the magnifying power of a microscope.—The magnifying power of a microscope is determined by comparing the distance between the lines of the stage micrometer, as they

FIG. 33.



Ocular-micrometer, natural size.

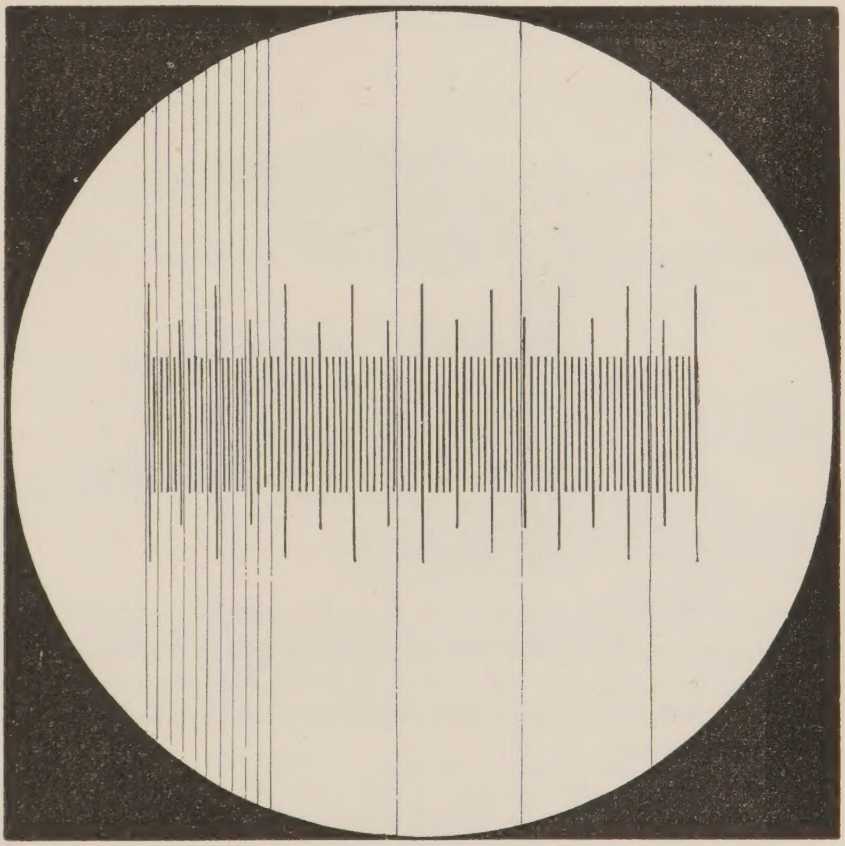
Part of the side is represented as broken away to show the field-glass at the bottom, and the micrometer-glass, *m*, a little below the middle. The collar, *r*, serves to vary the distance of the eye-glass from the micrometer.

appear imaged upon the paper, when this is exactly *ten inches*[1] from the eye, with the known interval between them. For instance, if, with the high power objective and the ordinary ocular, the interval of $\frac{1}{1000}$th of an inch of the micrometer was represented on the paper by a space of half an inch, this interval is magnified as many times as the $\frac{1}{1000}$th of an inch will go into half an inch, that is to say, 500 times; and every other object under similar conditions is magnified to a like extent.

[1] The ordinary distance of distinct vision.

The enlargement thus obtained may be determined once for all for each objective, the same ocular being used and the tube being drawn out to the same extent—say, to the full length—in each case, and scales may be made representing the intervals between the micrometer lines under the different powers. For purposes of measurement it will then only be necessary to compare the projected image of an object with the scale which was made under like conditions, without again making use of the stage micrometer.

FIG. 34.



Lines of stage micrometer viewed with an ocular-micrometer.

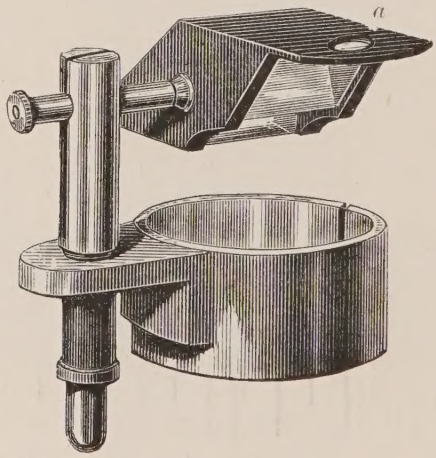
The finer lines are those of the stage micrometer ; about eighteen of the lines of the ocular micrometer are comprised in one of the larger intervals between them, so that, if these intervals represent $\frac{1}{100}$ inch, the subdivisions of the ocular micrometer will represent $\frac{1}{1800}$ inch.

Mode of drawing microscopic objects.—The most convenient and ready way of delineating an object is to sketch it with the free hand on paper placed at the right of the microscope, the left eye being applied to the tube. But if perfect accuracy of size

and relations of parts are desired, the outlines are first traced with a camera lucida. The simplest for ordinary use is that of Zeiss (fig. 35), which is a combination of two prisms so arranged that when placed above the ocular the surface of the table in front of the microscope is seen at the same time as the object, the image of the one being superposed on that of the other. The paper for drawing is placed on this part of the table, supported upon an inclined plane of wood, and the main outlines are traced out on it, the details and shading being afterwards filled up without the camera.

The lines of the stage micrometer can very easily be traced in the same way by aid of the camera lucida.

FIG. 35.



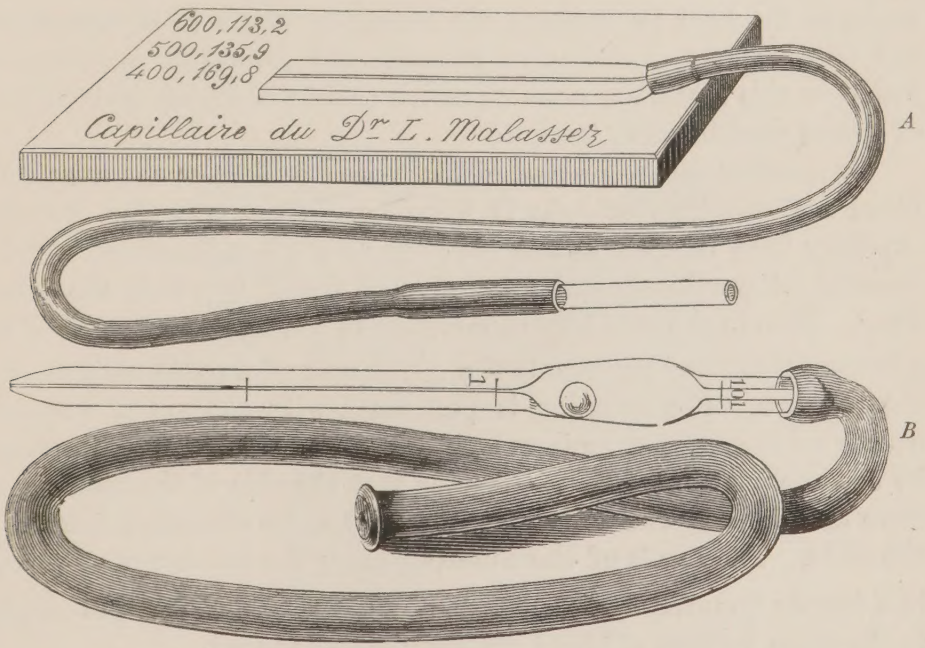
Camera lucida, for tracing the outlines of an object without tilting or otherwise disturbing the microscope. The metal ring fits on to the upper end of the microscope tube, and the aperture, *a*, is placed immediately over the eye-glass, this part of the camera being somewhat more depressed than is represented in the figure.

Mode of counting the blood-corpuscles.—In order to separate the corpuscles and prevent coagulation, the blood used is first diluted to a definite extent—say a hundred times—with a 10 per cent. solution of sulphate of soda. The mixing can be performed in a measuring glass if the blood is in sufficient quantity, but if only a small drop is obtainable, such, for example, as is got by pricking the finger, the mixer shown in fig. 36, *B*, may be used. This consists of a capillary tube terminating in a bulb, the capacity of the bulb between the marks 1 and 101 being exactly 100 times that of the tube from its point to the mark 1. A small glass ball is enclosed in the bulb, and

serves by its movements to facilitate the mixing. The capillary tube is allowed to fill with blood as far as the mark 1; sulphate of soda solution is then sucked up as far as the mark 101. As it passes in, it of course pushes the blood before it into the bulb, and the two are there thoroughly mixed by gentle agitation.

The next thing is to count the corpuscles in a known quantity of the mixture. The most convenient plan is that of Hayem and Nachet. A slide is used having a glass ring, $\frac{1}{5}$ millimeter in depth, cemented on to its upper surface. A drop of the mixture, not enough

FIG. 36.



Malassez's apparatus for counting the blood-corpuscles.

A, Capillary tube for receiving the diluted blood; *B*, pipette for mixing a small quantity of blood with the diluting fluid.

to fill the cell so formed, is placed in the middle of the ring, and a perfectly flat cover-glass is so laid on that the drop touches and adheres to it without reaching the sides of the cell. The slide is placed on the microscope, and as soon as the corpuscles have settled down to the bottom of the drop the number in a definite area is counted. If the area chosen is $\frac{1}{5}$th of a millimeter square, this will give the number which were contained in $\frac{1}{5}$th millimeter cube of the mixture, and multiplying this by the number of times the blood was diluted, the result will be the number of corpuscles in $\frac{1}{5}$th millimeter cube of blood.

Areas of $\frac{1}{5}$ millimeter square might be marked with a diamond (like the lines of a stage micrometer) upon the centre of the slide, and subdivided into smaller squares to facilitate the counting. But it is found more convenient to measure them off by using an ocular micrometer similar to the one shown in fig. 33, but with a large square subdivided into smaller squares substituted for the scale on the micrometer glass, *m*. To obtain the desired value for the large square, a stage micrometer, ruled in parts of a millimeter, is placed under the microscope, and by adjusting the draw-tube, the sides of the square on the ocular are made exactly to subtend the interval of $\frac{1}{5}$ millimeter on the stage micrometer. A mark is then made on the tube to indicate the extent to which it is drawn out, and whenever an examination is made the draw-tube is always adjusted to this mark, the same objective (one of moderate power) being of course used in every case.

By another method—that of Malassez—a little of the mixture of blood and sulphate of soda is transferred to a very fine flattened capillary tube (fig. 36 *A*) the capacity of a given length of which is ascertained previously and marked on the slide to which the tube is fixed. Thus in the capillary tube shown in the figure, a length of 400 micro-millimeters [1] represents the $\frac{1}{169{\cdot}8}$th part of a cubic millimeter of the mixture. The counting is performed with the aid of a squared ocular micrometer, the microscope as before having been so arranged by observation of a stage micrometer that the side of the square shall have the value of one of the lengths (400 μ, for example) marked on the slide. The result of the counting gives the number of corpuscles in a known quantity ($\frac{1}{169{\cdot}8}$ cub. mill.) of the mixture, and the number in the same volume of blood can readily be deduced.

Microtomes.—When it is desired to obtain a number of perfectly even, consecutive sections of an organ, of the spinal cord for instance, a section-instrument or microtome may be employed. One of the first of these to be introduced was that devised by Mr. Stirling (fig. 37), and most of the other instruments are modelled on the same principle. It consists of a hollow brass cylinder with a broad metal plate (*p*) fitted over the top, and a long, finely cut screw (*s*) working in the lower end, and serving to push a brass plug upwards in the tube. The instrument is clamped at the edge of the table by the screw *cs*.

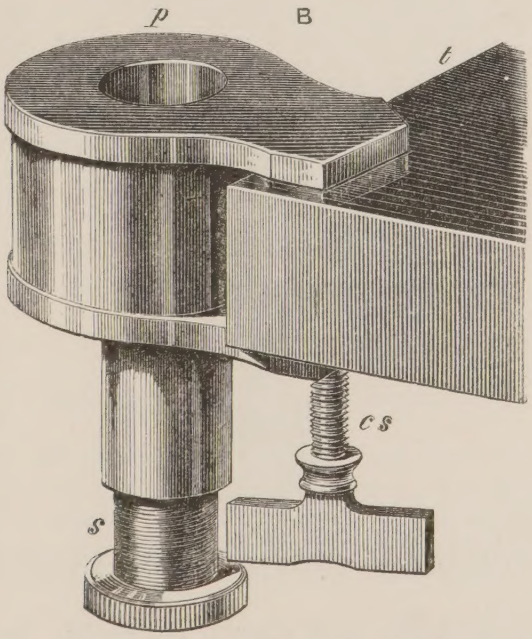
The tissue to be cut is embedded in the brass cylinder in much the same way as in an ordinary embedding trough. A little of the

[1] A micro-millimeter (μ) is the one-thousandth part of a millimeter.

embedding mass is first poured in; when this has begun to set the tissue is placed upon it, and the cylinder is then filled up with more embedding mass so that the object is completely enclosed. It is better to use a mixture of paraffin and hog's lard (5 parts paraffin to 1 part lard) as an embedding mass, since the ordinary wax and oil mixture tends in cooling to shrink away from the sides of the tube.

In cutting the sections the metal plate *p* serves to direct the razor, so that it moves in a perfectly even plane. By turning the screw *s* the plug of embedding mass with the included tissue is caused to project very slightly above the plane of the plate, so that when the razor, wetted as usual with spirit, is carried over this, a section is

Fig. 37.



Stirling's microtome.

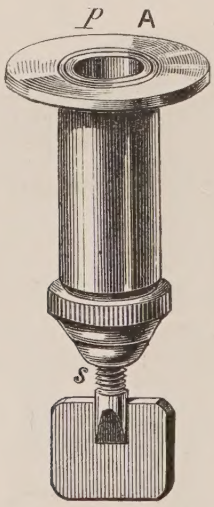
obtained varying in thickness according to the extent to which the screw was turned. In like manner a large number of consecutive slices may be made. But it is doubtful whether, with this or any other microtome, it is possible to get sections as thin as they can be made with the free hand.

Figure 38 shows a smaller instrument (Ranvier's), differing from the other one chiefly in the fact that it is held in the hand whilst the sections are cut, in place of being clamped to the table. Its smaller size renders it more handy to work with, and if it is necessary to

intermit the slicing for any length of time, the embedded tissue can be preserved without injury by inverting the instrument into a beaker of spirit.

Dr. Rutherford's freezing microtome (fig. 39) is designed to enable sections to be made of a tissue hardened by freezing. The instrument can also be used as an ordinary microtome. It may be described as an improved form of Stirling's microtome with the addition of a capacious metal trough which surrounds the upper part of the cylinder. The trough is filled with a freezing mixture (snow or pounded ice and salt, equal parts). Into the well or tube of the microtome is poured a thick solution of gum, which soon begins to freeze at the periphery. The tissue to be cut (which may be either fresh, or hardened by one

Fig. 38.



Simple form of microtome for holding in the hand whilst cutting sections.

of the ordinary methods, and should preferably be soaked in gum for some hours previously), is placed in the gum and held there until fixed by the advancing congelation, and when the whole is uniformly hard, sections are cut as with the ordinary instrument, except that the razor used must be dry, not wetted with spirit. The sections are placed in weak spirit (1 part spirit to 2 parts water) if of a hardened tissue, in salt solution if fresh, to dissolve out the gum, and they can then be mounted in any desired manner.[1]

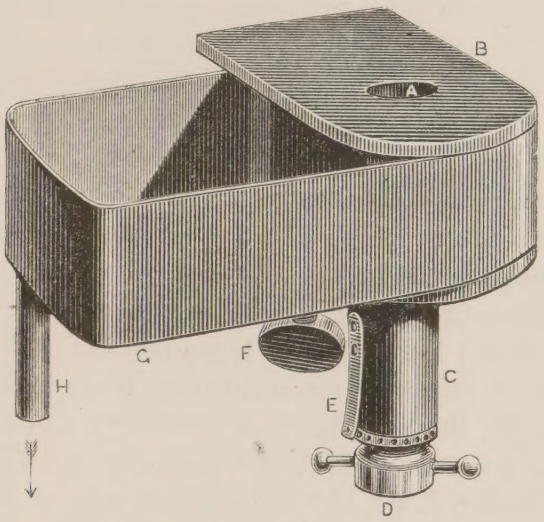
Gum is useful for the freezing process because it acquires a cheesy

[1] For a more complete description see Rutherford, 'Outlines of Practical Histology,' 2nd edition, 1876.

consistence when frozen, in place of becoming hard and crystalline (Urban Pritchard).

Microphotographic Apparatus.—Photography is every day coming more into use for obtaining images of microscopic objects. The mode of application consists in the adjustment of a photographic camera to the tube of the microscope (which it is better to divest of its eye-piece); the image formed by the objective is received and

FIG. 39.



Rutherford's freezing microtome.

B, Plate of gun-metal, with aperture, *A*, leading into well of microtome; *C*, lower end of cylinder in which the screw, *D*, works, moving a brass plug up or down; *E*, indicator for showing the fraction of a revolution imparted to the screw; *G*, trough to hold the freezing mixture, with *H*, tube to conduct away the water produced by the melting of the ice; *F*, clamp to fix the instrument to a table.

focussed upon the ground-glass plate of the camera, the objective of the microscope representing in fact the lens of the ordinary camera. Either wet or dry sensitized plates are employed, and the mode of exposing the plate and all the subsequent processes of developing, fixing, and printing are in every respect the same as in ordinary photography. It will be sufficient, therefore, in this place to describe a convenient form of apparatus (fig. 40), and the manner of obtaining an image of the microscopic object; an account of the subsequent procedure, and the precautions it is necessary to take, will be found in recognised standard works on photography.

Since it may often be required to photograph wet preparations,

the microscope should be kept vertical, and the camera must therefor be supported above the microscope. This is done by means of a strong mahogany frame consisting of a stand, *st*, upon which the microscope is placed and four vertical pillars rigidly connected below to the stand, and above to one another by crosspieces. Two brass rods, *r*, one on either side, run vertically between the stand below and the crosspieces above, and serve more immediately to support the camera, *c*, which is capable of sliding up and down on the rods and can be fixed by screw-clamps at any height.

The camera should be lightly constructed, with a bellows-adjustment gradually tapering, and fitted below with a cloth-lined tube which exactly fits over the microscope tube, to which it is clamped by the screw *d*. Just above the tube is placed the 'stop' for shutting the light off at any moment from the camera plate; it consists of a light hinged flap, which, by turning a small handle (not shown in the figure), may be made to fall over the aperture.

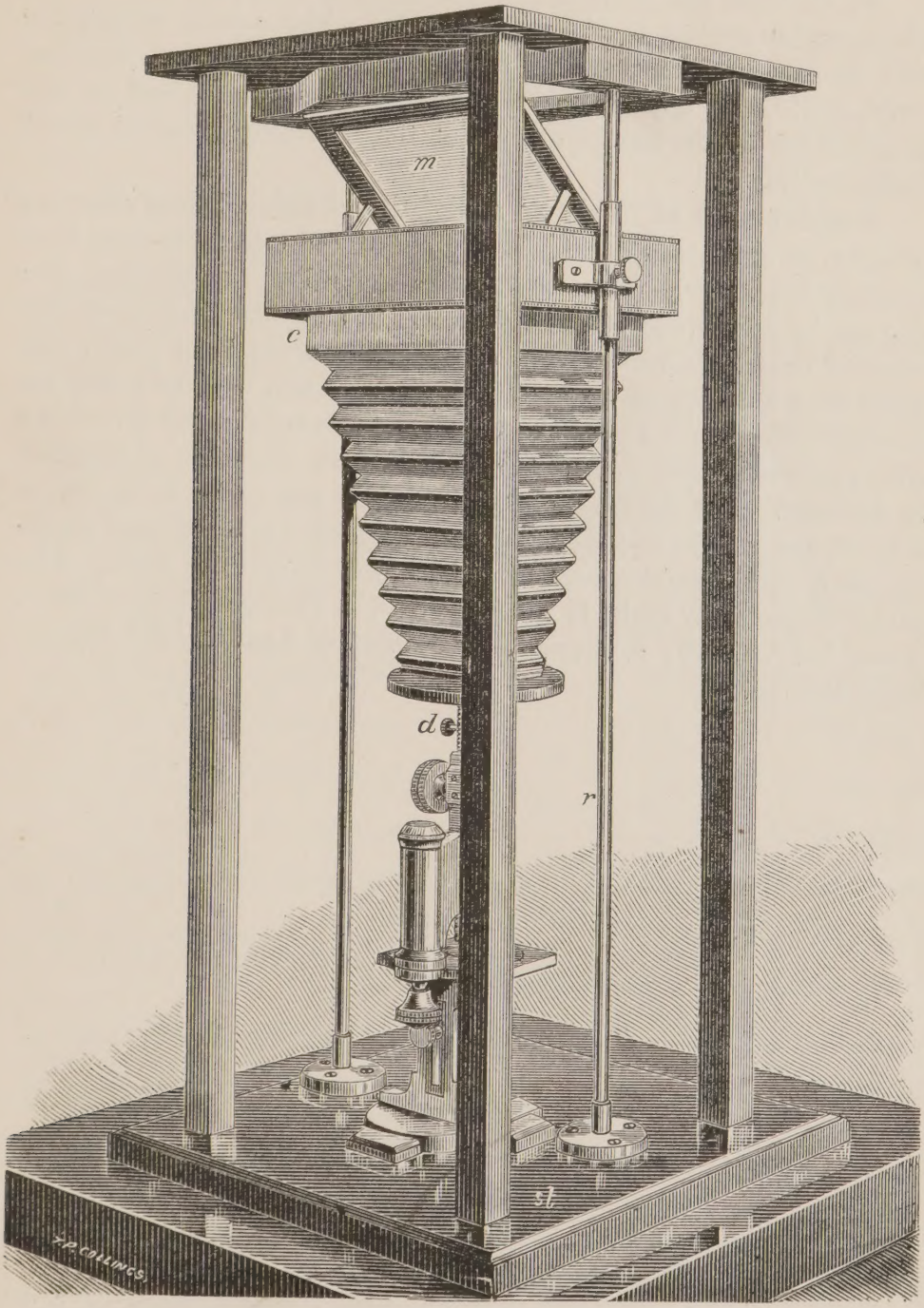
The microscope is put in the centre of the stand, being held firmly in place by three projecting blocks provided with strong wooden buttons for clamping the foot, and there is a round hole in the centre of the stand *st*, so that the apparatus can, if desired, be used in the horizontal position, the light being sent directly through the hole. It is well to unscrew and remove the whole of the upper part of the microscope tube (fig. 1. *t'*), the cloth-lined tube at the lower end of the camera being made to fit over the lower and larger part (*t*).

When the apparatus is in the position shown in the figure, the ground-glass camera plate is horizontal, and it would be extremely awkward to lean over and observe the image upon the plate and at the same time adjust the focus. To obviate this difficulty the plane mirror *m* is provided; it is inclined at a convenient angle, so that the image on the plate is reflected towards the observer standing behind the microscope. When focussing, the upper part of the apparatus as well as the head of the observer may be enveloped in a loose black cloth.

It is necessary to employ bright sunlight (or some other intense and actinic source of light), which should be allowed to pass through a glass vessel containing a solution of ammonio-sulphate of copper, so as to render the light mono-chromatic. It is then received on the mirror of the microscope (supposing the apparatus to be vertical) either directly or after traversing a condensing lens, and is reflected by the mirror up through the object and the tube of the microscope in the ordinary way. An objective of any magnifying

power, even an immersion, may be employed, provided there be light enough. With the same objective greater or less magnification is obtained according as the camera is raised or lowered.

FIG. 40.



Microphotographic apparatus. One-seventh the natural size.

The object is focussed upon the ground-glass with the utmost exactitude. The light is then cut off, the mirror, *m*, removed, and the sensitized plate substituted for the ground-glass plate as in the ordinary process.

Mr. G. Giles has devised a simple mode of adapting an ordinary photographic camera to the microscope, of which a brief description will be found in the 'Quarterly Journal of Microscopical Science,' 1876, p. 111. It appears to have yielded good results, but for wet preparations has the disadvantage that the microscope must be placed horizontally.

Employment of Eosin as a staining fluid.—Dilute solutions of eosin, an aniline-preparation newly introduced into commerce, have recently been much recommended for colouring the tissues. The dye can be used dissolved either in water or alcohol. For the watery solution Dreschfeld recommends a strength of about 1 per 1000; this takes from a minute to a minute and a-half to stain sections; they are subsequently put for a very short time into water slightly acidulated with acetic acid, and then either examined in glycerine or mounted in dammar. For portions of tissue which are to be hardened in alcohol, the process of hardening and staining can be effected simultaneously by the employment of an alcoholic solution of eosin. The colour imparted by eosin is a rose-red. (E. Fischer, 'Arch. f. Micr. Anat.' 1875, p. 349; J. Dreschfeld, 'Journal of Anatomy and Physiology,' Oct. 1876.)

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